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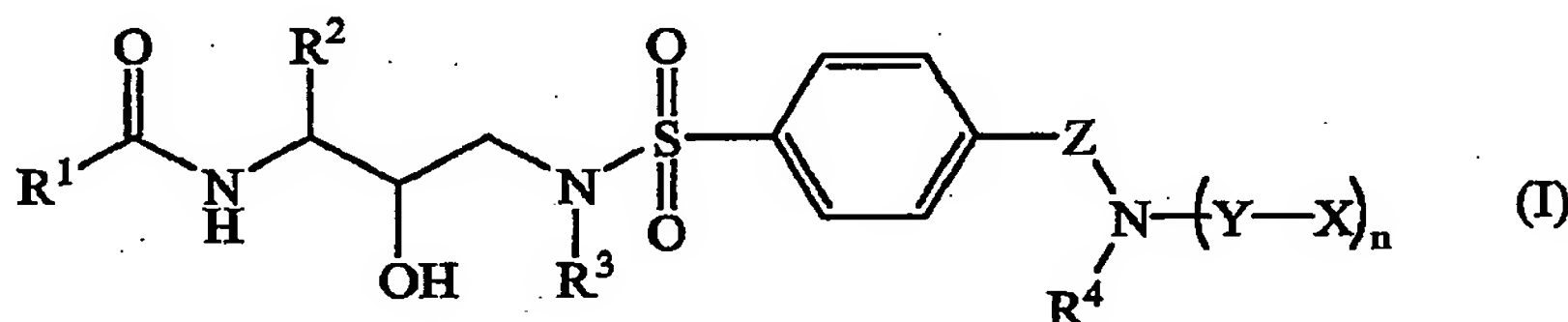
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(54) Title: HIV PRODRUGS CLEAVABLE BY CD26



(57) Abstract: The present invention provides new prodrugs which are conjugates of a therapeutic compound and a peptide wherein the conjugate is cleavable by dipeptidyl-peptidases, more preferably by CD26, also known as DPPIV (dipeptidyl aminodipeptidase IV). The present prodrugs have the formula (I), the stereoisomeric forms and salts thereof, wherein n is 1 to 5; Y is proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydroproline, pipecolic acid (L-homoproline), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine; X is selected from any amino acid in the D- or L-configuration; X and Y in each repeat of [Y-X] are chosen independently from one another and independently from other repeats; Z is a direct bond or a bivalent straight or branched saturated hydrocarbon group having from 1 to 4 carbon atoms; R¹ is an aryl, heteroaryl, aryloxy, heteroaryloxy, aryloxyC₁₋₄alkyl, heterocycloalkyloxy, heterocycloalkylC₁₋₄alkoxy, heteroaryloxyC₁₋₄alkyl, heteroarylC₁₋₄alkyloxy; R² is arylC₁₋₄alkyl; R³ is C₁₋₁₀alkyl, C₂₋₆alkenyl or C₃₋₇cycloalkylC₁₋₄alkyl; R⁴ is hydrogen or C₁₋₄alkyl. The present invention furthermore provides the use of said prodrugs as medicines as well as a method of producing said prodrugs.



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- 1 -

HIV PRODRUGS CLEAVABLE BY CD26**FIELD OF THE INVENTION**

The invention relates to prodrug of HIV inhibitory compounds whereby the HIV inhibitory compound is released or activated by proteolysis of a peptidic moiety. The invention also relates to methods for increasing oral uptake, modify serum protein binding, blood-brain barrier penetration or solubility and bioavailability of the HIV inhibitory compounds.

BACKGROUND OF THE INVENTION

Modern drug discovery techniques (e.g. combinatorial chemistry, high-throughput pharmacological screening, structure-based drug design) are providing very specific and potent drug molecules. However, it is rather common that these novel chemical structures have unfavourable physicochemical and biopharmaceutical properties. Besides, during the development of new therapeutic agents, researchers typically focus on pharmacological and/or biological properties, with less concern for physicochemical properties. However, the physicochemical properties (dissociation constant, solubility, partition coefficient, stability) of a drug molecule have a significant effect on its pharmaceutical and biopharmaceutical behaviour. Thus, the physicochemical properties need to be determined and modified, if needed, during drug development. Moreover, the physicochemical properties of many existing drug molecules already on the market are not optimal.

Today, drug candidates are often discontinued due to issues of poor water solubility or inadequate absorption, leaving countless medical advances unrealized. Still other products make it to the market, but never realize their full commercial potential due to safety or efficacy concerns. Prodrugs have the potential to overcome both challenges. The technology exploits endogenous enzymes for selective bioconversion of the prodrug to the active form of the drug. This technology has the ability to keep promising new drug candidates alive through development, and improving the safety and efficacy of existing drug products.

Prodrugs are mostly inactive derivatives of a drug molecule that require a chemical or enzymatic biotransformation in order to release the active parent drug in the body. Prodrugs are designed to overcome an undesirable property of a drug. As such this technology can be applied to improve the physicochemical, biopharmaceutical and/or pharmacokinetic properties of various drugs. Usually, the prodrug as such is biologically inactive. Therefore, prodrugs need to be efficiently converted to the parent drugs to reach pronounced efficacy as soon as the drug target has been reached.

-2-

In general, prodrugs are designed to improve the penetration of a drug across biological membranes in order to obtain improved drug absorption, to prolong duration of action of a drug (slow release of the parent drug from a prodrug, decreased first-pass metabolism of the drug), to target the drug action (e.g. brain or tumour targeting), to
5 improve aqueous solubility and stability of a drug (i.v. preparations, eye-drops, etc.), to improve topical drug delivery (e.g. dermal and ocular drug delivery), to improve the chemical / enzymatic stability of a drug (e.g. peptides) or to decrease drug side-effects.

Many prodrug technologies have already been developed depending on the kind of
10 drug that has to be converted. These prodrug technologies include cyclic prodrug chemistry for peptides and peptidomimetics, phosphonooxymethyl (POM) chemistry for the solubilization of tertiary amines, phenols and hindered alcohols and esterification in general. Also targeting strategies are pursued by coupling groups cleavable by specific enzymes such as the peptide deformylase of bacteria which
15 cleaves N-terminal formyl groups of the peptides or PSA (prostate specific antigen) used to target prostate cancer.

Coupling of peptides or amino acids to a therapeutic agent has already been pursued in the past for several reasons. In the antisense-antigene field, oligonucleotides or
20 intercalators have been conjugated to peptides in order to increase the cellular uptake of the therapeutic agents. These oligonucleotides and intercalators have not to be released after cell penetration however, and can not be regarded as prodrugs. An example of amino acid coupling to a therapeutic compound is Valgancyclovir, the L-valyl ester prodrug of gancyclovir, which is used for the prevention and treatment of
25 cytomegalovirus infections. After oral administration, the prodrug is rapidly converted to gancyclovir by intestinal and hepatic esterases. Recently, alanine and lysine prodrugs of novel anti-tumour benzothiazoles have been investigated. Peptide carrier-mediated membrane transport of amino acid ester prodrugs of nucleoside analogues has already been demonstrated [Han et al. *Pharm. Res.* (1998) 15: 1154-1159; Han et al *Pharm.*
30 *Res.* (1998) 15: 1382-1386]. It has indeed been shown that oral bioavailability of drugs can be mediated by amino acid prodrug derivatives containing an amino acid, preferably in the L-configuration. L-Valine seems to have the optimal combination of chain length and branching at the β -carbon of the amino acid for intestinal absorption. hPEPT-1 has been found to be implicated as the primary absorption pathway of
35 increased systemic delivery of L-valine ester prodrugs. Recently, it was shown that the hPEPT-1 transporter need to optimally interact with a free NH_2 , a carbonyl group and a lipophylic entity, and may form a few additional H-bridges with its target molecule. L-Valine-linked nucleoside analogue esters may fulfill these requirements for efficient

-3-

hPEPT-1 substrate activity [Friedrichsen et al. *Eur. J. Pharm. Sci.* (2002) 16: 1-13].

The prior art for ameliorating solubility and bioavailability reveals however only amino acid prodrugs (only one amino acid coupled) of small organic molecules whereby the amino acid is mostly coupled through ester bonds, since they are easily converted back to the free therapeutic agent by esterases.

Prior art documents describe processing of prodrugs by a number of proteases, such as aminopeptidases (PCT application WO01/68145) and aminotripeptidase (PCT application WO02/00263). PCT application WO99/67278 describes a targeting strategy for CD26/DPPIV inhibitors which become active upon processing by CD26/DPPIV and subsequently inactivate the protease.

There is however still a need for new, alternative and better prodrug technologies and this need is projected to grow, as combinatorial chemistry and high throughput screening continue to produce vast numbers of new compounds with a high molecular weight, high log P [partition coefficient], or poor water solubility.

SUMMARY OF THE INVENTION

The invention provides a novel prodrug technology that can be applied to ameliorate the solubility and/or the bioavailability of a therapeutic compound. The invention comprises the derivatization of therapeutic compounds in order to ameliorate their solubility and/or bioavailability. The invention provides conjugates of therapeutic compounds with a peptidic moiety wherein said conjugate is cleavable by a dipeptidyl-peptidase, such as CD26. This technology can furthermore be used to modulate the protein binding of a therapeutic compound D and to target specific sites in a mammal. Specific therapeutic compounds are HIV inhibitory compounds, in particular HIV protease inhibitory compounds, more in particular compounds of formula (Ia).

The present invention provides a new prodrug technology and new prodrugs in order to modulate the solubility, protein binding and/or the bioavailability of a drug. In the present invention the prodrugs are conjugates of a therapeutic compound D and a peptide wherein the conjugate is cleavable by dipeptidyl-peptidases, more preferably by dipeptidyl-peptidase IV. The present invention furthermore provides a method of producing said prodrugs. The invention also provides a prodrug technology to more selectively target drugs, to modify, particularly enhance brain and lymphatic delivery of drugs and/or to extend drug half-lives in plasma.

In one aspect the invention relates to a pharmaceutical composition comprising a prodrug of a therapeutic compound D. The therapeutic compound D is not a peptide or a protein, and includes a terminal primary or secondary amino-group capable of

-4-

binding with the carboxyl-group of an amino acid. Or alternatively, the therapeutic compound D is bound to a linker A_m comprising a primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid. The prodrug is characterised in that said prodrug comprises said therapeutic compound D linked to an oligopeptide, said oligopeptide consisting of a general structure $H-[X-Y]_n$, wherein n is between 1 and 5, wherein X and Y in each repeat of $[X-Y]$ are chosen independently from one another and independently from other repeats, and wherein X is an amino acid, and Y is an L amino acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydropoline, pipecolic acid (L-homoprolin), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy-terminus of $H-[X-Y]_n$ and the amino-group of D or its linker A_m occur via an amide. The $H-[X-Y]_n$ peptide has a free amino-terminus, i.e an unmodified NH_2 group. In one embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

In one embodiment the peptide has between two to five CD26 cleavable repeats. In another embodiment the CD26 cleavable oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide wherein at least one X is an hydrophobic or aromatic amino acid or alternatively, wherein at least one X is an neutral or acidic amino acid, or alternatively, wherein at least one X is a basic amino acid. In a particular embodiment the oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide selected from the group of Val-Y- $[X-Y]_{1-2}$, more in particular Val-Pro- $[X-Y]_{1-2}$ in order to have a good intestinal absorption, followed by a slow or fast release of the therapeutic compound combined with modifications of solubility, depending on the choice of X and Y. In one embodiment the tetra or hexapeptide has a general structure Val-Y- $[X-Y]$ or Val-Y- $[X-Y]_2$.

According to one embodiment Y is proline or hydroxyproline or dihydroxyproline or alanine. According to another embodiment, X is selected from Valine, Aspartic acid, Serine, Lysine, Arginine, Histidine, Phenylalanine, Isoleucine or Leucine. According to another embodiment, X is selected from the acidic amino acids Aspartic acid or Glutamic acid in order to have a slow cleavage, from the positively charged amino acids Arginine, Histidine or Lysine in order to have a fast release of the therapeutic compound D.

-5-

The oligopeptide $[X-Y]_n$ may be coupled via an amide binding to an amino group residing on an organic molecule/atom such as an aromatic group of a therapeutic compound, residing on a carbohydrate or residing on a nucleoside or on a heterocyclic group or residing on an alkyl, alkenyl or alkynyl or residing on an anorganic molecule/atom.

In one embodiment the oligopeptide $H-[X-Y]_n$ is coupled via an amide binding to an amino group residing on a aromatic group of a therapeutic compound, residing on a carbohydrate or residing on a nucleoside. Alternatively, the oligopeptide $H-[X-Y]_n$ is indirectly coupled to the therapeutic compound D via a linker comprising an amino group. Such a linker can have the general structure of an oligopeptide A_m wherein m ranges between 1 to 15 and more particularly between 1 to 3, or $m=1$. A in the structure A_m can be any amino acid. According to one embodiment $m=1$ and A is valine. A prodrug with such a linker has a general structure $H-[X-Y]_n-A_m-D$. The oligopeptide A_m or the amino acid A is linked at its amino-terminus via an amide binding to the oligopeptide $H-[X-Y]_n$. The oligopeptide A_m or the amino acid A is linked at its carboxy-terminus via an amide or ester binding to the therapeutic compound D. Pharmaceutical compositions can comprise prodrugs of drugs for the prevention or treatment of a viral infection. In one embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

In another aspect, the invention relates to a prodrug construct of a therapeutic compound D, wherein said therapeutic compound D is not a peptide or a protein, and wherein the therapeutic compound D includes a terminal primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid or wherein the therapeutic compound D is bound to a linker comprising a primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid, said prodrug consisting of said therapeutic compound D linked to an oligopeptide with a general structure $H-[X-Y]_n$, and is characterized in that $n = 1$ to 5, wherein X and Y in each repeat of $[X-Y]$ are chosen independently from one another and independently from other repeats, and wherein X is an amino acid, and Y is an L-amino acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thiopline), dehydroproline, pipecolic acid (L-homoproline), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy-terminus of $H-[X-Y]_n$ and the amino-group of D occurs via an amide. In one embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of

-6-

HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

According to one embodiment this prodrug, upon activation, has no inhibitory effect on the CD26/DPPIV enzyme. In one embodiment, the oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide wherein at least one X is a hydrophobic or aromatic amino acid, alternatively wherein at least one X is a neutral or acidic amino acid or, alternatively, wherein at least one X is a basic amino acid. In a particular embodiment the oligopeptide $H-[X-Y]_n$ is built with [X-Y] repeats selected from the group of Val-Pro, Asp-Pro, Ser-Pro, Lys-Pro, Arg-Pro, His-Pro, Phe-Pro, Ile-Pro, Leu-Pro, Val-Ala, Asp-Ala, Ser-Ala, Lys-Ala, Arg-Ala, His-Ala, Phe-Ala, Ile-Ala and Leu-Ala. According to one embodiment, Y is proline or hydroxyproline or dihydroxyproline or alanine.

According to one embodiment, the oligopeptide $[X-Y]_n$ is coupled via an amide binding to an amino group residing on a aromatic group of a therapeutic compound, residing on a carbohydrate or residing on a nucleoside. Alternatively, the oligopeptide $[X-Y]_n$ is indirectly coupled to the therapeutic compound D via a linker comprising an amino group. This linker comprises an organic molecule (i.e. alkylamino, a peptide, or a combination of both). In an embodiment, the number m of amino acids in the linker between the CD26 cleavable oligopeptide and the therapeutic compound D is between 1 and 15. In a particular embodiment, such a linker can have the general structure of an oligopeptide A_m wherein m ranges between 1 to 15 and more particularly between 1 to 3, or $m=1$. A in the structure A_m can be any amino acid. According to one embodiment $m=1$ and A is valine. A prodrug with such a linker has a general structure $H-[X-Y]_n-A_m-D$. According to one embodiment, the prodrug is a prodrug of a therapeutic compound for the prevention or treatment of a viral infection. According to another embodiment the prodrug is a HIV protease inhibitor prodrug with a general structure of formula (I).

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In another aspect the invention relates to a method for modulating (increasing or decreasing) the water solubility, and/or plasma protein binding and/or the bioavailability of a therapeutic compound D by coupling a peptide to said therapeutic compound whereby the resulting conjugate is cleavable by a dipeptidyl-peptidase.

According to one embodiment the dipeptidyl peptidase is CD26 and the therapeutic compound D is not a peptide or a protein, and the therapeutic compound D includes a terminal primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid or the therapeutic compound D is bound to a linker comprising a

-7-

primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid, and wherein the oligopeptide consists of a general structure $H-[X-Y]_n$, wherein n is between 1 and 5, wherein X and Y in each repeat of $H-[X-Y]$ are chosen independently from one another and independently from other repeats, and wherein X is an amino acid, and Y is an L amino acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thiopline), dehydroproline, pipecolic acid (L-homoproline), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy terminus of $H-[X-Y]_n$ and the amino-group of D occurs via an amide. According to one embodiment, the oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide wherein at least one X is a hydrophobic or aromatic amino acid, alternatively wherein at least one X is a neutral or acidic amino acid or, alternatively, wherein at least one X is a basic amino acid. In one embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

Another aspect of the invention relates to a method of producing a prodrug, wherein the prodrug is cleavable by a dipeptidyl-peptidase, the method comprising the step of linking a therapeutically active drug D and a peptide with structure $H-[X-Y]_n$ whereby the resulting conjugate is cleavable by CD26. According to one embodiment the dipeptidyl peptidase is CD26 and the therapeutic compound D is not a peptide or a protein, and the therapeutic compound D includes a terminal primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid or the therapeutic compound D is bound to a linker comprising a primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid, and wherein the oligopeptide consists of a general structure $H-[X-Y]_n$, wherein n is between 1 and 5, wherein X and Y in each repeat of $[X-Y]$ are chosen independently from one another and independently from other repeats, and wherein X is an amino acid, and Y is an L-amino acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thiopline), dehydroproline, pipecolic acid (L-homoproline), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy-terminus of $H-[X-Y]_n$ and the amino-group of D occurs via an amide. According to one embodiment, the oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide wherein at least one X is a hydrophobic or aromatic amino acid, alternatively wherein at least one X is a neutral or acidic amino acid or, alternatively, wherein at least one X is a basic amino acid. In one embodiment the therapeutic

-8-

compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

5 In another aspect, the present invention relates to the use of a prodrug of a therapeutic compound D for the manufacture of a medicament for the treatment or prevention of a viral infection. The therapeutic compound D is not a peptide or a protein, and the therapeutic compound D includes a terminal primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid or the therapeutic compound D is bound to a linker comprising a primary or secondary amino-group
10 capable of binding with the carboxylgroup of an amino acid, and characterised in that said prodrug comprises said therapeutic compound D linked to an oligopeptide, said oligopeptide consisting of a general structure $H-[X-Y]_n$, wherein n is between 1 and 5, wherein X and Y in each repeat of $[X-Y]$ are chosen independently from one another and independently from other repeats, wherein X is an amino acid, and Y is an L amino
15 acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydroproline, pipercolic acid (L-homoprolin), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy-terminus of $H-[XY]_n$ and the amino-group of D occurs via an amide. In one
20 embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

Yet another aspect of the invention relates to a manufacturing process for the
25 production of prodrugs using a peptide with general structure $H-[X-Y]_n$ for the preparation of a CD26 cleavable prodrug of a therapeutic compound D. The therapeutic compound D is not a peptide or a protein, and the therapeutic compound D includes a terminal primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid or alternatively the therapeutic compound D is bound to a linker
30 comprising a primary or secondary amino-group capable of binding with the carboxyl-group of an amino acid The prodrug is characterised in that said prodrug comprises said therapeutic compound D linked to an oligopeptide, said oligopeptide consisting of a general structure $H-[X-Y]_n$, wherein n is between 1 and 5, wherein X and Y in each repeat of $[X-Y]$ are chosen independently from one another and independently from
35 other repeats, and wherein X is an amino acid, and Y is an L amino acid selected from the group consisting of proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydroproline, pipercolic acid (L-homoprolin), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine,

valine, leucine, isoleucine and threonine, and wherein the binding between the carboxy-terminus of $H-[X-Y]_n$ and the amino-group of D or its linker occur via an amide. In one embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the
5 formula (I).

In one embodiment the peptide has between two to five CD26 cleavable repeats. In another embodiment, the number m of amino acids in the linker A_m between the CD26 cleavable oligopeptide and the therapeutic compound is 1 and A is valine. In another
10 embodiment to CD26 cleavable oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide wherein at least one X is an hydrophobic or aromatic amino acid or alternatively, wherein at least one X is an neutral or acidic amino acid, or alternatively, wherein at least one X is a basic amino acid. In a particular embodiment the oligopeptide $H-[X-Y]_n$ is a tetrapeptide or hexapeptide selected from the group of Val-Pro- $[X-Y]_{1-2}$
15 in order to have a good intestinal absorption, followed by a slow or fast release of the therapeutic compound, depending on the choice of X. Within a prodrug construct $H-[X-Y]_n-D$, the therapeutic compound D has a primary (NH_2) or secondary (NH) amino group which is bound to the COOH group of the carboxy-terminal amino acid of the $H-[X-Y]_n$ peptide. When the therapeutic compound D has no NH_2 or NH group, or the
20 NH or NH_2 group can not react (due e.g. steric hindrance). The therapeutic compound D can be reacted with a linker which, after reaction has a NH_2 or NH group, which can react with the COOH group of the carboxy-terminal amino acid of the $H-[X-Y]_n$ peptide.

25 According to one embodiment each Y independently is proline or hydroxyproline or dihydroxyproline or alanine. In one embodiment the oligopeptide $H-[X-Y]_n$ is coupled via an amide binding to an amino group residing on a aromatic group of a therapeutic compound, residing on a carbohydrate or residing on a nucleoside. Alternatively, the oligopeptide oligopeptide $H-[X-Y]_n$ is indirectly coupled to the therapeutic compound
30 D via a linker comprising one or amino group. Such a linker can have the general structure of an oligopeptide A_m wherein m ranges between 1 to 15 and more particularly between 1 to 3, or $m=1$. A in the structure A_m can be any amino acid. According to one embodiment $m=1$ and A is valine. A prodrug which such a linker has a general structure $H-[X-Y]_n-A_m-D$. The oligopeptide A_m or the amino acid A is linked
35 at its amino-terminus via an amide binding to the oligopeptide $H-[X-Y]_n$. The oligopeptide A_m or the amino acid A is linked at its carboxy-terminus via an amide or ester binding to the therapeutic compound D. Pharmaceutical compositions can comprise prodrugs of drugs for the prevention or treatment of viral infection. In one

embodiment the therapeutic compound is an antiviral drug, in particular an inhibitor of HIV protease, more in particular a compound of formula (Ia) and the prodrug has the formula (I).

5 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the inhibitory effect of different concentrations of the dipeptide Val-Pro against CD26-catalysed conversion of the chromophoric substrate GP-pNA (25 μ M) to GP + pNA at 5 (left bar), 10 (middle bar) or 15 min (right bar) of reaction. The CD26 catalytic reaction was measured by recording the increase of absorption
10 caused by pNA release at 400 nm.

Figure 2 shows the conversion of Val-Pro-PI 1 prodrug to PI 1 (HIV protease inhibitor) in function of time. A: CD26; B: bovine serum; C: human serum (both 10% in PBS).

Figure 3 shows the conversion of Val-Pro-PI 1 prodrug to PI 1 (HIV protease inhibitor) in function of time. Figure 3a: Bovine serum (2% in PBS), figure 3b: Human
15 serum (2% in PBS).

Figure 4 shows the inhibitory (competitive) effect of Val-Pro-PI 1 on CD26-catalysed conversion of GP-pNa to GP+pNA (yellow).

Figure 5 shows inhibitory (competitive) effect of Val-Pro-PI 1 on CD26-catalysed
20 conversion of GPpNA to GP+pNA (yellow) in 2% human serum (in PBS).

Figure 6 shows inhibitory (competitive) effect of Val-Pro-PI 1 on CD26-catalysed conversion of GPpNA to GP+pNA (yellow) in 2% bovine serum (in PBS).

DETAILED DESCRIPTION OF THE INVENTION

25 The term "prodrug or prodrugs" as used herein refers to mostly inactive derivatives (or derivatives with strongly reduced activity, i.e. less than 20 %, less than 10%, less than 5% or even less than 1% residual activity of the underived drug molecule) of a therapeutic compound that require a chemical or enzymatic transformation in order to release the active parent drug. The prodrug of the present invention has a general
30 structure H-[X-Y]_n-D. The chemical nature of this prodrug is explained in detail below. Prodrugs are designed to overcome an undesirable property of a drug. As such this technology can be applied to improve the physicochemical, biopharmaceutical and/or pharmacokinetical properties of various drugs. Usually, the prodrug as such is biologically inactive. Therefore, prodrugs need to be efficiently converted to the parent
35 drugs to reach pronounced efficacy as soon as the drug target has been reached. This

-11-

activation can be done by enzymes, which are present in the body, alternatively the enzymes are co-administrated with the prodrug.

5 In general, prodrugs are designed to improve the penetration of a drug across biological membranes in order to obtain improved drug absorption, to prolong duration of action of a drug (slow release of the parent drug from a prodrug, decreased first-pass metabolism of the drug), to target the drug action (e.g. brain or tumor targeting), to improve aqueous solubility and stability of a drug (i.v. preparations, eyedrops, etc.), to improve topical drug delivery (e.g. dermal and ocular drug delivery), to improve the
10 chemical / enzymatic stability of a drug (e.g. peptides) or to decrease drug side-effects.

The term "therapeutic compound D" as used herein refers to any agent having a beneficial effect on a disease, any agent that is or will be used in the future as a therapy for a certain disease or disorder. This refers also to all molecules which are still in the
15 discovery or development phase and which have not proven their efficacy and safety yet. This includes small organic molecules, proteins, peptides, oligonucleotides, carbohydrates, aliphatic carbon chains, aromatic compounds and analogues and derivatives.

20 The therapeutic compound D with a (terminal) amino group, more in particular a primary or secondary amino group, refers to therapeutic compounds with a free amino group (primary or secondary), namely a NHR group, wherein R can be hydrogen (primary) or any other chemical group known in the art. The amino group can be coupled to the therapeutic compound D via a saturated or unsaturated carbon, to
25 carbonyl, or can be part of other broader functionalities (amide, carbamate, etc.) wherein the amino group is comprised, but the amino group in each circumstance has to be able to react with an amino acid in order to form stable amide bonds. In a particular embodiment, the amino group NHR of the therapeutic compound belongs to the functional group of amine functions and does not belong to a broader general
30 functionality such as amides or carbamates.

The therapeutic compound can also be linker to an oligopeptide through a linker. This linker can have any organic structure, thereby including amino acids, and contains a
35 NHR group as described above.

"CD26" as used herein refers to the dipeptidyl-peptidase IV (EC 3.4.14.5) in its membrane bound and free form. Synonyms for CD26 are DPPIV, DPP4, CD26/DPPIV or ADCP2 (adenosine deaminase complexing protein 2).

-12-

As used herein, "dipeptidyl-peptidase(s)" refers to enzymes with a dipeptidyl aminopeptidase activity, i.e removing a dipeptide from the aminoterminal side of a substrate side by cleavage of the second CO-NH amide bond in the substrate. Other enzymes than CD26 with a comparable activity and proteolytic specificity as CD26 (i.e. prolyl oligopeptidases) are referred to by "dipeptidyl-peptidase(s)". "Dipeptidyl-peptidase IV" refers to CD26.

As used herein, the term "peptide" or "oligopeptide" relates to two or more amino acids which are connected by amide bindings. When mentioned in conjunction with a therapeutic compound D, the peptide or oligopeptide refers to two or more amino acids which are connected by an amide binding, originating from a COOH group of the peptide and a NH₂ or NH group on the therapeutic compound D or a linker connected to the therapeutic compound. The length of a peptide is indicated by Greek numbers preceding the word -peptide (dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, etc.).

In the present invention, a new prodrug technology is provided based on the coupling of a peptide to a therapeutic agent, whereby the amide bond of the conjugates is cleavable by a dipeptidyl-peptidase, such as CD26. As such, the solubility, bioavailability and the efficacy of the therapeutic compound D in general can be modulated more extensively. The lymphocyte surface glycoprotein CD26 belongs to a unique class of membrane-associated peptidases. It is characterized by an array of diverse functional properties and it is identical to dipeptidyl-peptidase IV (DPP IV, EC 3.4.14.5). DPP IV is a member of the prolyl oligopeptidase (POP; EC3.4.21.26) family, a group of atypical serine proteinases able to hydrolyze the prolyl bond. The 766-amino acid long CD26 is anchored to the cellular lipid bilayer membrane by a single hydrophobic segment, and has a short cytoplasmic tail of six amino acids [Abbott et al. *Immunogenetics* (1994) 40: 331-338]. The membrane anchor is linked to a large extracellular glycosylated region, a cysteine-rich region and a C-terminal catalytic domain (Abott et al. cited supra). CD26 is strongly expressed on epithelial cells (i.e. kidney proximal tubules, intestine) and on several types of endothelial cells and fibroblasts, as well as leukocyte subsets [Hegen, M. In: *Leukocyte Typing VI*. Kishimoto, T., ed. Garland Publishing, (1997), pp. 478-481]. CD26 also occurs as a soluble form present in seminal fluids, plasma and cerebrospinal fluid. It lacks the intracellular tail and the transmembrane region [De Meester et al. *Rev. Immunol. Today* (1999) 20: 367-375]. In addition to its exopeptidase activity, CD26 specifically binds to several proteins outside its substrate-binding site [i.e. adenosine deaminase [Trugnan et al. In: *Cell-Surface Peptidases in Health and Disease*. Kenny, & Boustead., eds. BIOS,

-13-

(1997), pp. 203-217], fibronectin [Gonzalez-Gronow, et al. *Fibrinolysis* (1996), 10 (Suppl. 3):32], collagen [Löster et al. *Biochem. Biophys. Res. Commun.* (1995), 217: 341-348]. CD26 is endowed with an interesting (dipeptidyl) peptidase catalytic activity and it has a high selectivity for peptides with a proline or alanine at the penultimate
5 position of the N-terminus of a variety of natural peptides.

Several cytokines, hematopoietic growth factors, neuropeptides and hormones share the X-Pro or X-Ala motif at their N-terminus and have been shown to act as efficient substrates for the enzyme [reviewed in De Meester et al. *Rev. Immunol. Today* (1999)
10 20: 367-375 and Mentlein *Regul. Pept.* (1999) 85: 9-24]. Substance P is even an example of a natural peptide of 11 amino acids containing an Arg-Pro-Lys-Pro [SEQ ID NO:1] sequence at its H-terminus, and which is cleaved by CD26 to an active heptapeptide by stepwise release of Arg-Pro and Lys-Pro [Ahmad et al. *Pharmacol. Exp. Ther.* (1992), 260: 1257-1261]. CD26 can cut dipeptides from very small natural
15 peptides [i.e. the pentapeptide enterostatin (Val-Pro-Asp-Pro-Arg) [SEQ ID NO:2] [Bouras et al. *Peptides* (1995), 16: 399-405] to larger peptides [including the chemokines RANTES and SDF-1 α and IP-10 (68 to 77 amino acids)] containing respectively the Ser-Pro, Lys-Pro and Val-Pro sequences at their amino terminus [Oravecz et al. *J. Exp. Med.* (1997), 186: 1865-1872; Proost et al. *J. Biol. Chem.*
20 (1998), 273:7222-7227; Ohtsuki et al. *FEBS Lett.* (1998), 431: 236-240 ; Proost et al. *FEBS Lett.* (1998), 432: 73-76].

Although a relatively restricted substrate specificity (penultimate Pro or Ala) has been observed for CD26, lower cleavage rates have also sometimes been observed when the
25 penultimate amino acids were Gly, Ser, Val or Leu instead of Pro or Ala (De Meester et al. cited supra). Also, the nature of the terminal amino acid plays a role in the eventual catalytic efficiency of CD26. There is a decreasing preference from hydrophobic (i.e. aliphatic: Val, Ile, Leu, Met and aromatic Phe, Tyr, Trp) to basic (i.e. Lys, Arg, His) to neutral (i.e. Gly, Ala, Thr, Cys Pro, Ser, Gln, 7Asn) to acidic (i.e. Asp, Glu) amino
30 acids as the preferred first amino acid at the amino terminus for efficient cutting of the peptide by CD26 (De Meester et al. cited supra). Also unnatural amino acids are recognised. The observation that a double truncation of macrophage-derived chemokine (MDC) by CD26 can occur thereby sequentially losing Gly¹-Pro² followed by Tyr³-Gly⁴, suggests that the substrate activity of CD26 may be less restricted to the
35 penultimate Pro or Ala than generally accepted [Proost, P. et al. *J. Biol. Chem.* (1999), 274: 3988-3993].

- Many other hydrolases (EC 3), more specifically peptidases (EC 3.4) and yet more specifically aminopeptidases (EC 3.4.11) such as prolyl aminopeptidase (EC 3.4.11.5) and X-Pro aminopeptidase (EC 3.4.11.9) have already been identified. Also other dipeptidases (EC 3.4.13), peptidyl-dipeptidases (EC 3.4.15) and dipeptidyl-peptidases (EC 3.4.14, this EC-group also includes tripeptidyl-peptidases) exist next to CD26.
- 5 Dipeptidyl-peptidase I (EC 3.4.14.1) occurs in the lysosome and cleaves a dipeptide from a peptide with consensus sequence $X_1-X_2-X_3$ except when X_1 is Arg or Lys or X_2 or X_3 is Pro. Dipeptidyl-peptidase II (EC 3.4.14.2) is a lysosomal peptidase that is maximally active at acidic pH and releases dipeptides from oligopeptides
- 10 (preferentially tripeptides) with a sequence $X_1-X_2-X_3$ wherein X_2 preferably is Ala or Pro. DPP III (EC 3.4.14.4) is a cytosolic peptidase and cleaves dipeptides from a peptide comprising four or more residues dipeptidyl-dipeptidase (EC 3.4.14.6). X-Pro dipeptidyl-peptidase (EC 3.4.14.11) is a microbial peptidase with similar activity to CD26. Some of them are found in humans and other mammals, while others are
- 15 produced by micro-organisms such as yeast and fungi. They differ in first instance in amino acid sequence, but also in their specificity for recognizing amino acid sequences. In, addition, database screening with DPPIV revealed novel proline specific dipeptidases (DPP8, DPP9, DPP10) [Qi et al. *Biochem J.* (2003) 373,179-189]. Most of these proline specific dipeptidases occur intracellularly in the lysosome and act at
- 20 acidic pH. Only DPPIV occurs as a membrane bound protein at the outside of a cell or as a secreted protein. Thus according to one embodiment, the compounds of the present invention are cleavable by an extracellular or membrane bound dipeptidyl peptidase at neutral pH.
- 25 The present invention demonstrates that peptidyl prodrug derivatives are efficiently converted to the parent compound by the exodipeptidyl-peptidase activity of CD26. The present invention further demonstrates that the peptidyl prodrug derivatives are extracellularly processed to the parent therapeutic compound.
- 30 Since an L-valine moiety can be involved in the dipeptidyl prodrug approach, this technology may represent a powerful tool to make lipophilic compounds not only markedly more water-soluble and less protein binding, but also to enhance oral bioavailability and plasma delivery of the parent molecule and a more selective delivery of the parent drug to CD26-expressing cells.
- 35 In view of this observation, the present invention provides a new prodrug technology in order to modulate the solubility, plasma protein binding and/or to enhance the bioavailability of a drug. In other embodiments of the invention, prodrugs are delivered

-15-

in order to more selectively target drugs, to enhance brain and lymphatic delivery of drugs and/or to extend drug half-lives in plasma. The present invention provides new prodrugs, characterized in that the prodrugs are cleavable by the dipeptidyl-peptidase CD26 or other enzymes with the same activity and proteolytic specificity as CD26. In a preferred embodiment, the prodrugs of the present invention are peptide-drug conjugates and derivatives thereof, that include amino acid sequences containing cleavage sites for dipeptidyl-peptidases, such as CD26. As such, the invention also provides a therapeutic prodrug composition comprising a therapeutic compound D linked to a peptide via an amide bond, which is specifically cleaved by dipeptidyl-peptidases, such as CD26.

The therapeutic compound D can be linked to the carboxy group of an amino acid either directly or through a linker group. In a preferred embodiment, the therapeutic compound D and the peptide are directly coupled via an amide bond. The therapeutic compound D can have a free amino group (primary or secondary) amide that can be coupled with the carboxyl group of amino acids, more preferably with the α -carboxyl group. In another preferred embodiment, the therapeutic compound D and the peptide are coupled via a linker, wherein the linker can be of non-peptidic or peptidic nature. If the connection between the therapeutic compound D and the peptide is made through a linker, the connection between the linker and the first amino acid is preferably an amide bond. The linker may be connected to the therapeutic compound D through any bond types and chemical groups known to those skilled in the art, more preferably by covalent bonding. The linker may remain on the therapeutic compound D indefinitely after cleavage, or may be removed thereafter, either by further reactions with agents present in the mammal or in a self-cleaving step. External agents which may affect cleavage of the linker include enzymes, proteins, organic or inorganic reagents, protons and other agents. In embodiments in which the linker remains attached to the drug, the linker can be any group which does not substantially inhibit the activity of the drug after cleavage of the peptide. In other embodiments, the linker is self-cleaving. Self-cleaving linkers are those which are disposed to cleave from the drug after the cleavage of the peptide by dipeptidyl-peptidases, such as CD26. Mechanisms involved in the self-cleavage of the linkers are for example intra molecular cyclisation or spontaneous S_N1 solvolysis and release the drug upon peptide cleavage. Some examples of linkers are provided in Atwell et al. (Atwell et al. *J. Med. Chem.* 1994, 37: 371-380). The linkers generally contain primary amines which form amide bonds to the carboxy terminus of the peptide. The linkers can also contain a carboxylic acid which forms an amide bond to a primary amine found on the drug. The linker can be coupled to the

-16-

drug by one or more reactions chosen from the reactions available to the person skilled in the art.

In one embodiment the protease which can be used for proteolysis of the prodrug is CD26. The obtained experimental data reveal that CD26 relies for its cleavage only on the dipeptide structure. Its activity is not hampered by the presence of the therapeutic compound D immediately after the amide bond between proline and the drug moiety. In the same context, there is thus no need to have additional peptide or other linker molecules between the dipeptide drug and Furthermore, due to its tissue expression (on both cancer and normal tissue) on different organs (from high level to lower levels: kidney, lung, adrenal gland, jejunum, liver, glandula parotis, spleen, testis and also on skin, heart, pancreas, brain, spinal cord, serum), and different cell types (such as thymocytes, endothelial cells, lymphocytes, microglial cells), several applications and several therapeutic applications can be envisaged. The rate of proteolysis of a dipeptide can be modulated by modifying the amino-terminal amino acid and/or the second amino acid. Together or independently of the modulation of hydrolysis, the physicochemical character of the dipeptide prodrug can be modified.

The therapeutic compounds that may be used in the prodrugs of the invention include any drugs (except from protein or peptide drugs such as peptide hormones) that can be directly or indirectly linked to a peptide and whereby the conjugate is cleavable by a dipeptidyl-peptidase, such as CD26. In addition to known therapeutic compounds, this invention can also be applied to the novel drug molecules that are currently under drug development or to drug molecules which are already in clinical use. In another preferred embodiment, the therapeutic compound D is a small organic molecule and not a peptide, protein, an intercalator or an oligonucleotide or analogs thereof (such as HNA, PNA, etc.). The therapeutic molecule can have an activity in the cardiovascular, neurological, respiratory, oncology, metabolic diseases, immunology, urology, anti-infectives, inflammation and all other therapeutic fields. In yet another more preferred embodiment, the therapeutic compound D has an antiviral activity. In still a more preferred embodiment, the therapeutic compound D has an anti-HIV activity.

Preferred drugs are those containing primary amines. The presence of a primary amine allows the formation of an amide bond between the drug and the peptide. The primary amines may be found in the drugs as commonly provided, or they may be added to the drugs by chemical synthesis.

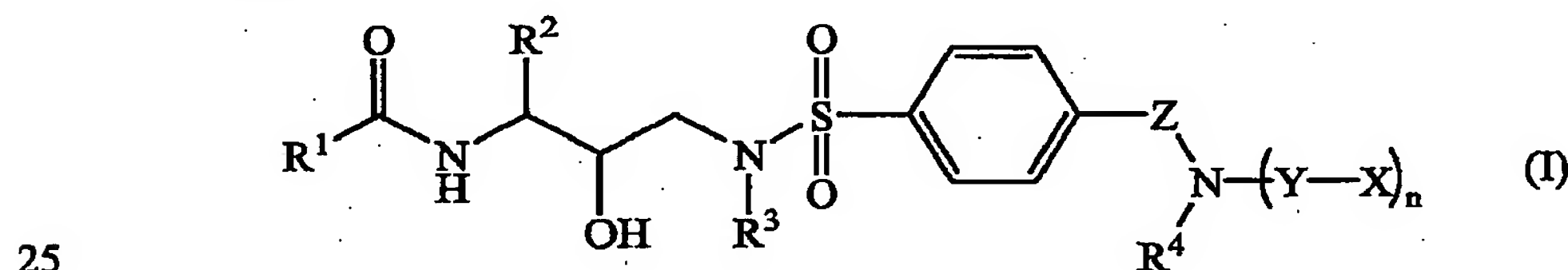
According to the FDA's Biopharmaceutics Classification System (BCS), drug substances are classified as follows: Class I - High Permeability, High Solubility; Class

-17-

II - High Permeability, Low Solubility; Class III - Low Permeability, High Solubility and Class IV - Low Permeability, Low Solubility. How drugs are classified in this classification system is described in the guidelines of the BCS. In a preferred embodiment, the therapeutic compounds D that can be used in the invention are
5 selected from class II, III and IV.

The invention provides for prodrugs that are cleavable by dipeptidyl-peptidases. The dipeptidyl-peptidases can be selected from the group of peptidases (EC 3.4) and yet more specifically aminopeptidases (EC 3.4.11) such as prolyl aminopeptidase (EC
10 3.4.11.5) and X-Pro aminopeptidase (EC 3.4.11.9), from the group of dipeptidases (EC 3.4.13), peptidyl-dipeptidases (EC 3.4.15) and dipeptidyl-peptidases (EC 3.4.14, this EC-group also includes tripeptidyl-peptidases) such as dipeptidyl-peptidase I (EC 3.4.14.1), II (EC 3.4.14.2), III (EC 3.4.14.4), IV (EC 3.4.14.5), dipeptidyl-dipeptidase (EC 3.4.14.6) and X-Pro dipeptidyl-peptidase (EC 3.4.14.11). In a preferred
15 embodiment, the prodrug is cleavable by dipeptidyl-peptidases present in mammals or more preferably in humans. In a more preferred embodiment, the prodrug is cleavable by dipeptidyl-peptidase IV (CD26), as well by the cell-surface bound as by the soluble form present in seminal fluids, plasma and cerebrospinal fluid. The occurrence of two different types of CD26 allows the application of prodrugs for activation at the cell
20 membrane and for activation in body fluids. It is another advantage of the present invention that prodrug can be

In one particular embodiment, the present invention relates to prodrug compounds of formula (I)



the stereoisomeric forms and salts thereof,

wherein

n is 1, 2, 3, 4 or 5;

Y is proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid
30 (thiopline), dehydroproline, pipecolic acid (L-homopline),
azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine,
isoleucine and threonine;

X is selected from any amino acid in the D- or L-configuration;

- X and Y in each repeat of [Y-X] are chosen independently from one another and independently from other repeats;
- Z is a direct bond or a bivalent straight or branched saturated hydrocarbon group having from 1 to 4 carbon atoms;
- 5 R^1 is an aryl, heteroaryl, aryloxy, heteroaryloxy, aryloxyC₁₋₄alkyl, heterocycloalkyloxy, heterocycloalkylC₁₋₄alkyloxy, heteroaryloxyC₁₋₄alkyl, heteroarylC₁₋₄alkyloxy;
- R^2 is arylC₁₋₄alkyl;
- R^3 is C₁₋₁₀alkyl, C₂₋₆alkenyl or C₃₋₇cycloalkylC₁₋₄alkyl;
- R^4 is hydrogen or C₁₋₄alkyl;
- 10 aryl, when used alone or in combination with another group, means phenyl optionally substituted with one or more substituents each individually selected from the group consisting of C₁₋₄alkyl, hydroxy, C₁₋₄alkyloxy, nitro, cyano, halo, amino, mono- or di(C₁₋₄alkyl)amino and amido;
- heteroaryl, when used alone or in combination with another group, means a monocyclic
- 15 or bicyclic aromatic heterocycle having one or more oxygen, sulphur or nitrogen heteroatoms, which aromatic heterocycle may optionally be substituted on one or more carbon atoms with a substituent selected from the group consisting of C₁₋₄alkyl, C₁₋₄alkyloxy, amino, hydroxy, aryl, amido, mono- or di(C₁₋₄alkyl)-amino, halo, nitro, heterocycloalkyl and C₁₋₄alkyloxycarbonyl, and which
- 20 aromatic heterocycle may also be optionally substituted on a secondary nitrogen atom by C₁₋₄alkyl or arylC₁₋₄alkyl;
- heterocycloalkyl, when used alone or in combination with another group, means a
- saturated or partially unsaturated monocyclic or bicyclic heterocycle having one
- or more oxygen, sulphur or nitrogen heteroatoms, which heterocycle may
- 25 optionally be substituted on one or more carbon atoms with a substituent selected from the group consisting of C₁₋₄alkyl, C₁₋₄alkyloxy, hydroxy, halo and oxo, and which heterocycle may also be optionally substituted on a secondary nitrogen atom by C₁₋₄alkyl or arylC₁₋₄alkyl.
- 30 The term C₁₋₄alkyl as a group or part of a group means straight and branched chained saturated monovalent hydrocarbon radicals containing from 1 to 4 carbon atoms. Examples of such C₁₋₄alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl and the like.
- 35 The term C₁₋₆alkyl as a group or part of a group means straight and branched chained saturated monovalent hydrocarbon radicals containing from 1 to 6 carbon atoms. Examples of such C₁₋₆alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl,

-19-

isobutyl, sec-butyl, tert-butyl, 2-methylbutyl, pentyl, iso-amyl, hexyl, 3-methylpentyl and the like.

5 The term C₁₋₁₀alkyl as a group or part of a group means straight and branched chained saturated monovalent hydrocarbon radicals containing from 1 to 10 carbon atoms. Examples of such C₁₋₁₀alkyl radicals include the examples of C₁₋₆alkyl radicals and heptyl, octyl, nonyl, decyl, 3-ethyl-heptyl and the like.

10 C₂₋₆alkenyl as a group or part of a group means straight and branched chained monovalent hydrocarbon radicals having at least one double bond and containing from 2 to 6 carbon atoms. Examples of such C₂₋₆alkenyl radicals include ethenyl, propenyl, 1-butenyl, 2-butenyl, isobutenyl, 2-methyl-1-butenyl, 1-pentenyl, 2-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 3-methyl-2-pentenyl and the like.

15 The term "halo" or "halogen", when used alone or in combination with another group, is generic to fluoro, chloro, bromo or iodo.

The term C₃₋₇cycloalkyl, when used alone or in combination with another group, is generic to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

20 For therapeutic use, the salts of the prodrug compounds of the present invention are those wherein the counter-ion is pharmaceutically or physiologically acceptable. However, salts having a pharmaceutically unacceptable counter-ion may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound of the present invention. All salts, whether pharmaceutically acceptable or
25 not are included within the ambit of the present invention.

The pharmaceutically acceptable or physiologically tolerable acid addition salt forms which the prodrug compounds of the present invention are able to form can conveniently be prepared using the appropriate acids, such as, for example, inorganic
30 acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic, malonic, succinic, maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like acids.

35

Conversely said acid addition salt forms can be converted by treatment with an appropriate base into the free base form.

-20-

The prodrug compounds of the present invention containing an acidic proton may also be converted into their non-toxic metal or amine addition salt form by treatment with appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, quaternary ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, N-methyl, -D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

Conversely said base addition salt forms can be converted by treatment with an appropriate acid into the free acid form.

The term "salts" also comprises the hydrates and the solvent addition forms that the prodrug compounds of the present invention are able to form. Examples of such forms are e.g. hydrates, alcoholates and the like. The term "salts" also comprises the quaternization of the nitrogen atoms of the present compounds. A basic nitrogen can be quaternized with any agent known to those of ordinary skill in the art including, for instance, lower alkyl halides, dialkyl sulfates, long chain halides and arylalkyl halides.

The present prodrug compounds may also exist in their tautomeric forms. Such forms, although not explicitly indicated in the above formula, are intended to be included within the scope of the present invention.

In one embodiment, the terminal amino group of the terminal amino acid of the peptide bond formed by $-(Y-X)_n$ may optionally contain one or two capping groups selected from acetyl, succinyl, benzyloxycarbonyl, glutaryl, morpholinocarbonyl and C_{1-4} alkyl.

In one embodiment, each X independently is selected from a naturally occurring amino acid.

In one embodiment, each X independently is an L-amino acid selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine.

In one embodiment, each Y independently is proline, alanine, glycine, serine, valine or leucine; preferably each Y independently is proline or alanine.

In one embodiment, n is 1, 2 or 3.

-21-

In one embodiment, $-(Y-X)_n$ is $-(Y-X)_{1\text{ or }2}$ -Y-Val, more in particular $-(Y-X)_n$ is $(Y-X)_{1\text{ or }2}$ -Pro-Val.

5 In one embodiment, the $(Y-X)_n$ oligopeptide is built up with $(Y-X)$ repeats selected from the group consisting of Pro-Val, Pro-Asp, Pro-Ser, Pro-Lys, Pro-Arg, Pro-His, Pro-Phe, Pro-Ile, Pro-Leu, Ala-Val, Ala-Asp, Ala-Ser, Ala-Lys, Ala-Arg, Ala-His, Ala-Phe, Ala-Ile and Ala-Leu.

10 In one embodiment, R^1 is heterocycloalkyloxy, heteroaryl, heteroaryl C_{1-4} alkyloxy, aryl or aryloxy C_{1-4} alkyl.

In one embodiment, R^1 is hexahydrofuro[2,3-b]furanyl-oxy, tetrahydrofuranyl-oxy, quinolinyl, thiazolylmethyloxy, aryl, aryloxymethyl.

15 In one embodiment, R^1 is hexahydrofuro[2,3-b]furan-3-yl-oxy, tetrahydrofuran-3-yl-oxy, quinolin-2-yl, thiazol-5-ylmethyloxy, 3-hydroxy-2-methyl-1-phenyl, 2,6-dimethyl-phenoxyethyl.

20 In one embodiment, R^1 is (3R, 3aS, 6aR)-hexahydrofuro[2,3-b]furan-3-yl-oxy, (3S)-tetrahydrofuran-3-yl-oxy, quinolin-2-yl, thiazol-5-ylmethyloxy, 3-hydroxy-2-methyl-1-phenyl, 2,6-dimethylphenoxyethyl.

Interesting groups of compounds are those groups of compounds of formula (I) thereof where one or more of the following restrictions apply :

- 25
- n is 1, 2 or 3;
 - Y is proline;
 - each X independently is selected from valine, aspartic acid, lysine or proline;
 - Z is methylene;
 - R^1 is heterocycloalkyloxy;
 - 30 • R^2 is phenylmethyl;
 - R^3 is C_{1-10} alkyl;
 - R^4 is hydrogen.

35 Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein R^2 is phenylmethyl.

Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein R^3 is C_{1-4} alkyl, in particular R^3 is isobutyl.

-22-

Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein R⁴ is hydrogen.

5 Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein R² is phenylmethyl; R³ is isobutyl and R⁴ is hydrogen.

Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein Z⁴ is methylene.

10 Interesting compounds are those compounds of formula (I) or any defined subgroup thereof wherein R¹ is hexahydrofuro[2,3-b]furanyl-oxy, tetrahydrofuranyl-oxy, quinolinyl, thiazolylmethyloxy, aryl, aryloxymethyl; R² is phenylmethyl; R³ is isobutyl and R⁴ is hydrogen.

15 A particular group of compounds are those compounds of formula (I) or any defined subgroup thereof wherein

n is 1, 2 or 3;

Y is proline or alanine;

each X independently is selected from a naturally occurring amino acid;

20 Z is a direct bond or methylene;

R¹ is heterocycloalkyloxy, heteroaryl, heteroarylC₁₋₄alkyloxy, aryl or aryloxyC₁₋₄alkyl;

R² is phenylmethyl;

R³ is isobutyl;

R⁴ is hydrogen.

25

Also a particular group of compounds are those compounds of formula (I) or any defined subgroup thereof wherein

n is 1, 2 or 3;

Y is proline;

30 each X independently is selected from a naturally occurring amino acid;

Z is methylene;

R¹ is hexahydrofuro[2,3-b]furanyl-oxy, tetrahydrofuranyl-oxy, quinolinyl, thiazolylmethyloxy, aryl, aryloxymethyl;

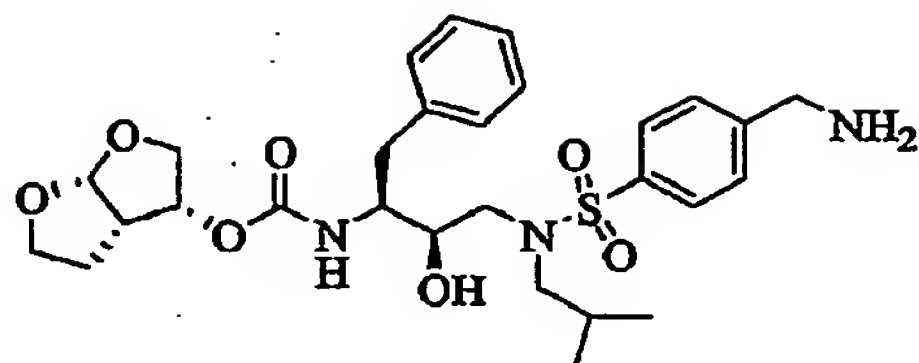
R² is phenylmethyl;

35 R³ is isobutyl;

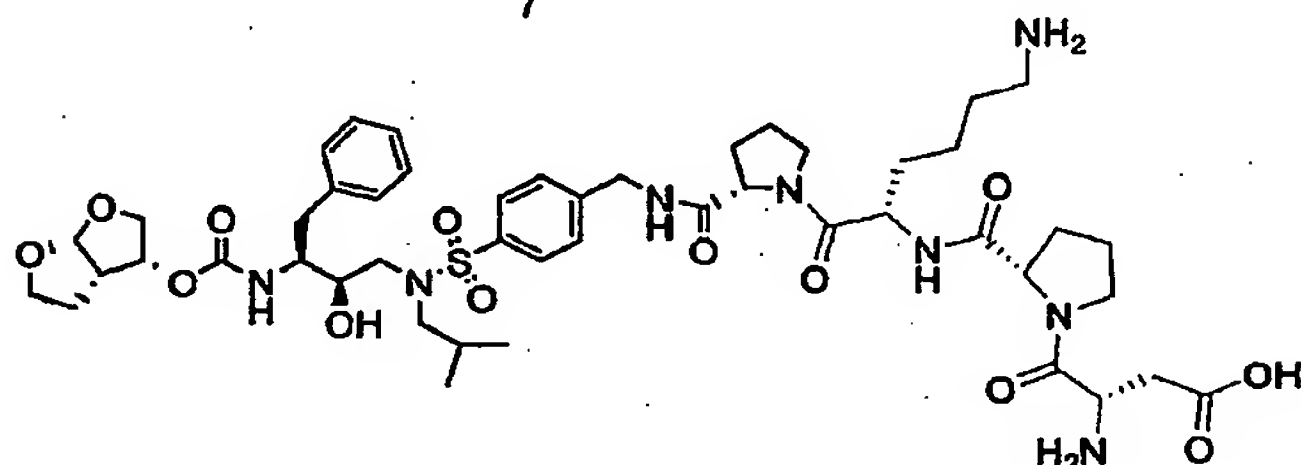
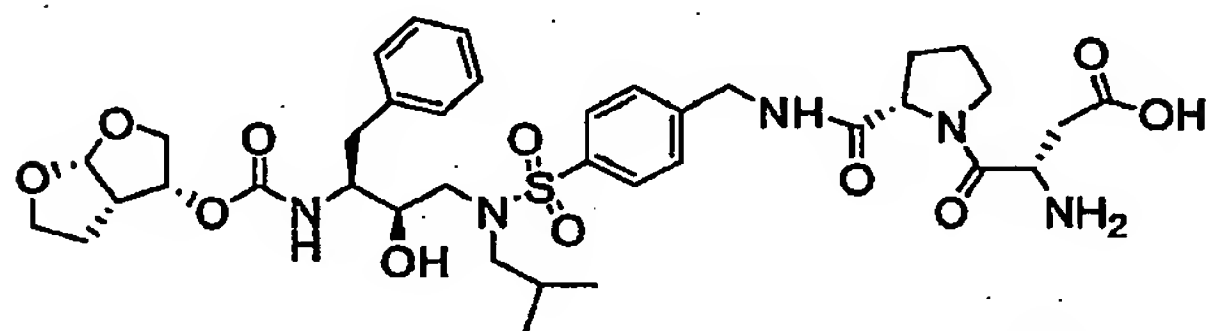
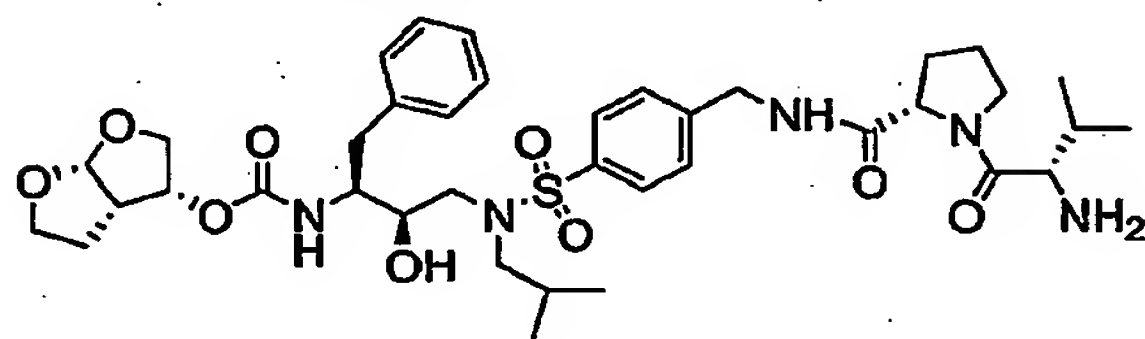
R⁴ is hydrogen.

Preferred prodrugs are those prodrugs wherein the therapeutic compound is

-23-



Preferred prodrugs include



5

and their salt forms.

Particularly, the amino-terminal end of the peptide in the prodrug comprises X-Pro, X-Ala, X-Gly, X-Ser, X-Val, or X-Leu, wherein X represents any amino acid or isomers (i.e. L- or D-configuration) thereof. Other dipeptides, with on the second position hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydroproline, pipecolic acid (L-Homoproline), azetidinecarboxylic acid, and aziridinecarboxylic acid are also cleavable by CD26. In a preferred embodiment, the peptide comprises aminoterminally X-proline or X-alanine. As such the amino acids can be selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and derivatives thereof. Also modified (i.e. hydroxylproline) or unnatural amino acids can be included. In another preferred embodiment, the length of the peptide is between 2 and 10 amino acids and can therefore have a length of 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. In another preferred embodiment, the peptide comprises $[X-Y]_n$ repeated units wherein X represents any amino acid, Y is selected from Pro, Ala, Gly, Ser, Val or Leu and n is

-24-

selected from 1, 2, 3, 4 or 5. In another more preferred embodiment, said peptide is a dipeptide. In still a more preferred embodiment, the dipeptide is Lys-Pro. In another still more preferred embodiment, the amino acids have the L-configuration. The amino-terminus of the peptide may also contain conventional capping groups. Such capping
 5 groups include acetyl, succinyl, benzyloxycarbonyl, glutaryl, morpholinocarbonyl, methyl and many others known in the art. Those skilled in the art can make substitutions to achieve peptides with better profile related to solubility, bioavailability and targeting of the conjugate. Therefore, the invention includes the peptide sequences as described above, as well as analogues or derivatives thereof, as long as the
 10 conjugates remain cleavable by dipeptidyl-peptidase, such as CD26.

In another embodiment the linker peptides of the present invention consist of one or more repetitive X-Y dipeptides with structure $[X-Y]_n$ which is cleavable by CD26 comprise one or more amino acids between the CD26 cleavable peptide and the
 15 prodrug and have a general structure $[X-Y]_n-A_m$. Herein A is any amino acid. The binding between the $[X-Y]_n$ oligopeptide and the consecutive A amino acid is an amide binding to allow CD26 proteolysis. The binding between two A amino acids and between an A amino acid and the prodrug can be either an amide binding or an ester binding. m can vary in length between 1 to 15. In one embodiment m is 1 and m can be
 20 hydrolysed from the prodrug by an esterase or an aminopeptidase.

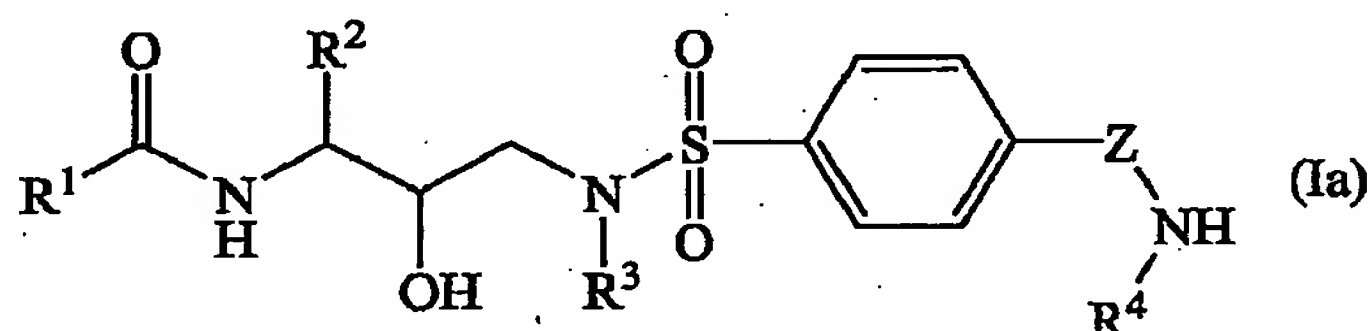
In another embodiment the CD26 cleavable oligopeptide $[X-Y]_n$ is a peptide wherein at least one X is an hydrophobic or aromatic amino acid or alternatively, wherein at least one X is an neutral or acidic amino acid, or alternatively, wherein at least one X is a
 25 basic amino acid. To modulate hydrophobicity and/or proteolysis rate of longer peptides (n is 3, 4, 5) more than one X will have the specific type of side chains to achieve the desired effect.

Also the choice of Y may influence the proteolysis rate, the hydrophobicity, solubility,
 30 bioavailability and the efficacy of the prodrug.

In yet another embodiment, the peptides with a general structure $[X-Y]_n$ are tetrapeptides or hexapeptides with a structure selected from the group of XF-Y-XF-Y, XF-Y-XS-Y, XS-Y-XF-Y, XS-Y-XS-Y, XB-Y-X-Y, X-Y-XB-Y and XB-Y-XB-Y or a
 35 hexapeptide with a structure selected from the group of XF-Y-XF-Y-XF-Y, XS-Y-XF-Y-XF-Y, XF-Y-XS-Y-XF-Y, XF-Y-XF-Y-XS-Y, XF-Y-XS-Y-XS-Y, XS-Y-XF-Y-XS-Y, XS-Y-XS-Y-XF-Y and XS-Y-XS-Y-XS-Y, XB-Y-X-Y-X-Y, XB-Y-XB-Y-X-Y, X-Y-XB-Y-XB-Y, XB-Y-X-Y-XB-Y and XB-Y-XB-Y-XB. Herein F stands for fast and XF is an amino acid that results in a rapid release of a dipeptide by CD26 (for

-25-

- example acidic and neutral amino acids such as Aspartic acid and Glutamic acid). Herein S stands for slow and XS is an amino acid that causes a slow release of a dipeptide by CD26 (for example charged and neutral amino acids). Herein B stands for basic and XB is a basic amino acid (Lys, Arg and His) leading to a moderate release of a charged and hydrophilic dipeptide. Such combinations allow tailor-made combinations of peptides that give a prodrug a well defined rate of degradation together with a defined hydrophobicity. For example the degradation of a hydrophobic prodrug with Tyr/Phe-Pro dipeptide can be delayed by the presence of an additional aminoterminal Gly-Pro dipeptide, resulting in a Gly-Pro-Tyr/Phe-Pro [SEQ ID:NO3] tetrapeptide prodrug. Hydrophobicity can even be increased by adding an additional Tyr/Phe-Pro dipeptide leading to the hexapeptide prodrug Gly-Pro-Tyr/Phe-Pro-Tyr/Phe-Pro [SEQ ID NO:4]. If a charged peptide prodrug with slow release is desired, Asp-Pro-Lys-Pro [SEQ ID NO:5] might be preferred over Gly-Pro. Other combinations can be developed by the skilled person wherein a tetrapeptide or hexapeptide allows the modulation of solubility and degradation rate of a peptide prodrug by CD26. For other purposes, proline can be replaced by alanine. The physicochemical properties and degradation rate of an undigested, partially digested and completely digested prodrug can be evaluated by determination of its retention time on reversed phase chromatography.
- The present invention demonstrates that peptidyl prodrug derivatives of formula (I) are efficiently converted to the parent therapeutic compound of formula (Ia)



- wherein R^1 , R^2 , R^3 , R^4 and Z are as defined in the compounds of formula (I); by the exodipeptidyl-peptidase activity of CD26,

- These therapeutic compounds of formula (Ia) are known to have HIV protease inhibiting activity and are described in EP656887, EP715618, EP810209, US5744481, US5786483, US5830897, US5843946, US5968942, US6046190, US6060476, US6248775, WO99/67417 all incorporated herein by reference.

- Due to the fact that the therapeutic compounds of formula (Ia) are inhibitors of the replication of HIV, the prodrug compounds of formula (I) are useful in the treatment of warm-blooded animals, in particular humans, infected with HIV. Conditions associated with HIV which may be prevented or treated with the compounds of the present

-26-

invention include AIDS, AIDS-related complex (ARC), progressive generalized lymphadenopathy (PGL), as well as chronic CNS diseases caused by retroviruses, such as, for example HIV mediated dementia and multiple sclerosis.

- 5 The prodrug compounds of the present invention may therefore be used as medicines against or in a method of treating above-mentioned conditions. Said use as a medicine or method of treatment comprises the systemic administration of an effective therapeutic amount of a compound of formula (I) to HIV-infected warm-blooded animals, in particular HIV-infected humans. Consequently, the prodrug compounds of
10 the present invention can be used in the manufacture of a medicament useful for treating conditions associated with HIV infection.

The term stereochemically isomeric forms of compounds of the present invention, as used hereinbefore, defines all possible compounds made up of the same atoms bonded
15 by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of the present invention may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound encompasses the mixture of all possible stereochemically isomeric forms which said compound may possess. Said mixture may contain all diastereomers and/or
20 enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of the present invention both in pure form or in admixture with each other are intended to be embraced within the scope of the present invention.

- 25 Pure stereoisomeric forms of the compounds and intermediates as mentioned herein are defined as isomers substantially free of other enantiomeric or diastereomeric forms of the same basic molecular structure of said compounds or intermediates. In particular, the term 'stereoisomerically pure' concerns compounds or intermediates having a stereoisomeric excess of at least 80% (i. e. minimum 80% of one isomer and maximum
30 20% of the other possible isomers) up to a stereoisomeric excess of 100% (i.e. 100% of one isomer and none of the other), more in particular, compounds or intermediates having a stereoisomeric excess of 90% up to 100%, even more in particular having a stereoisomeric excess of 94% up to 100% and most in particular having a stereoisomeric excess of 97% up to 100%. The terms 'enantiomerically pure' and
35 'diastereomerically pure' should be understood in a similar way, but then having regard to the enantiomeric excess and the diastereomeric excess respectively, of the mixture in question.

Pure stereoisomeric forms of the compounds and intermediates of this invention may be obtained by the application of art-known procedures. For instance, enantiomers may be separated from each other by the selective crystallization of their diastereomeric salts with optically active acids. Alternatively, enantiomers may be separated by
5 chromatographic techniques using chiral stationary phases. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably, if a specific stereoisomer is desired, said compound will be synthesized by stereospecific methods of preparation. These methods will
10 advantageously employ enantiomerically pure starting materials.

The diastereomeric racemates of the compounds of the present invention can be obtained separately by conventional methods. Appropriate physical separation methods which may advantageously be employed are, for example, selective
15 crystallization and chromatography, e.g. column chromatography.

The compounds may contain one or more asymmetric centres and thus may exist as different stereoisomeric forms. The absolute configuration of each asymmetric centre that may be present in the compounds may be indicated by the stereochemical
20 descriptors R and S, this R and S notation corresponding to the rules described in Pure Appl. Chem. 1976, 45, 11-30.

In one embodiment, the carbon atom bearing the R^2 substituent has the "S" configuration and the adjacent carbon atom bearing the hydroxy substituent has the "R"
25 configuration.

The present invention is also intended to include all isotopes of atoms occurring on the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of
30 hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.

The present invention furthermore provides a method of producing a prodrug of formula (I), wherein the prodrug is cleavable by a dipeptidyl-peptidase, such as CD26. This method of producing a prodrug of formula (I) comprises the step of linking a
35 therapeutically active drug of formula (Ia) and a peptide. In a more preferred embodiment, the therapeutically active drug or the peptide are in a first step derivatised in order to be able to link the therapeutic compound of formula (Ia) and the peptide in a later step via an amide binding. Many acceptable methods of coupling carboxyl and amino groups to form amide bindings are known to those skilled in the art.

-28-

In general, the therapeutic compounds of formula (Ia) can be prepared as described in EP656887, EP715618, EP810209, US5744481, US5786483, US5830897, US5843946, US5968942, US6046190, US6060476, US6248775, WO99/67417.

5

General peptide chemistry is straightforward for a person skilled in the art and involves coupling of natural amino acids in order to form a peptide. Several chemical strategies are available of which the fluorenylmethyloxycarbonyl (Fmoc) and tert-

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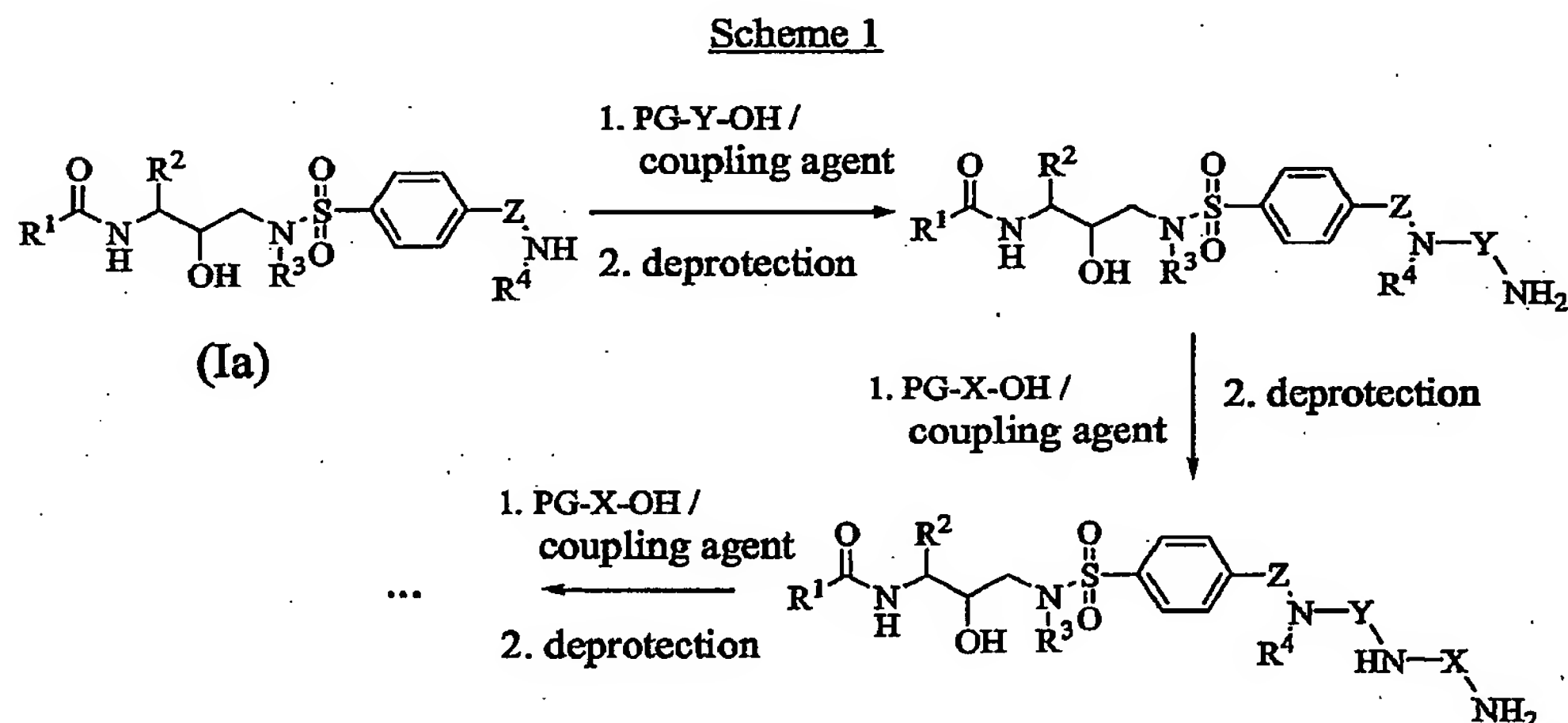
Butyloxycarbonyl (Boc) chemistry are the most widely used. Fields G.B. gives an extensive description of the peptide chemistry that can be applied to couple amino acids to each other or to a therapeutic compound D [Fieldsin *Methods in Molecular Biology, Vol. 35: Peptide Synthesis Protocols* Humana Press Inc.: Totawa, (1994), pp. 17 – 27]. Solid phase as well as solution phase chemistry can be applied [Atherton & Sheppard *Solid Phase Peptide Synthesis* IRL Press: Oxford-New York-Tokyo, (1989)].

15

Protection strategies whereby functionalities of a therapeutic compound that can not react during the prodrug preparation procedures are blocked through coupling of a protecting group, will have to be used.

20

More in particular, the prodrug compounds of formula (I) can be prepared starting from the therapeutic compounds of formula (Ia) using art-known peptide chemistry.



25 For instance, amino acids may be coupled to the therapeutic compound to form peptide bonds as depicted in scheme 1. This coupling reaction may be performed in an appropriate reaction-inert solvent such as N,N-dimethylformamide, acetonitrile, dichloromethane, tetrahydrofuran or any other solvent that solubilizes the reagents, with an amino protected amino acid of formula PG-Y-OH wherein PG (protecting

-29-

group) may be for instance a Boc (*tert*-butyl oxycarbonyl), Cbz (benzyloxycarbonyl) or Fmoc, in the presence of a coupling agent such as DCC (dicyclohexylcarbodiimide) or EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and HOAt (1-hydroxy-7-azabenzotriazol) or a functional equivalent thereof. The thus formed
5 peptide may then be deprotected using conventional deprotection techniques such as for instance deprotection with trifluoroacetic acid in dichloromethane.

This coupling and subsequent deprotection reaction step can be repeated using
10 PG-X-OH as reagent to form the desired peptide bond.

Some of the amino acids, such as for example lysine and aspartic acid may require a second protecting group and can be represented in formula PG-(XPG)-OH or PG-(YPG)-OH.

15 Alternatively, a reagent of formula PG-X-Y-OH, or PG-(X)_n-OH, or PG-(X-Y)_n-OH, can be used in the above reaction procedures.

In preparations presented above, the reaction products may be isolated from the reaction medium and, if necessary, further purified according to methodologies
20 generally known in the art such as, for example, extraction, crystallization, distillation, trituration and chromatography.

The compounds of formula (I) as prepared in the hereinabove described processes may be synthesized as a mixture of stereoisomeric forms, in particular in the form of
25 racemic mixtures of enantiomers which can be separated from one another following art-known resolution procedures. The racemic compounds of formula (I) may be converted into the corresponding diastereomeric salt forms by reaction with a suitable chiral acid. Said diastereomeric salt forms are subsequently separated, for example, by selective or fractional crystallization and the enantiomers are liberated therefrom by
30 alkali. An alternative manner of separating the enantiomeric forms of the compounds of formula (I) involves liquid chromatography using a chiral stationary phase. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably if a specific stereoisomer is desired, said
35 compound will be synthesized by stereospecific methods of preparation. These methods will advantageously employ enantiomerically pure starting materials.

-30-

Methods exist to predict the solubility of a compound. For example in J Chem Inf Comput Sci 1998 May-Jun;38(3):450-6 the aqueous solubility prediction of drugs based on molecular topology and neural network modelling has been described.

- 5 In fact, all parameters relevant for solubility and bioavailability (pKa, partition coefficient, etc.) can be determined. "Drug Bioavailability: Estimation of Solubility, Permeability, Absorption and Bioavailability" gives a comprehensive overview of these parameters and their determination or prediction (ISBN 352730438X).
- 10 Partition coefficients are a measurement of lipophilicity. Expressed numerically as 'log P' values, they are the ratios between the concentrations of substances in two immiscible phases, such as water/octanol or water/liposomes and they can be easily calculated. Substances with high log P values dissolve better in fats and oils than in water. This enhances their ability to enter lipid (fat-based) membranes in the body by
- 15 passive diffusion, thereby enhancing their potential for absorption.

- Many drugs have a log P value of between one and four, making them suitable for oral methods of delivery. Drugs with high log P are usually poorly soluble in water. They may be lipid-soluble, but they cannot dissolve in the GI tract, so can't diffuse into the
- 20 gut wall. If they do enter membranes, they may become trapped, with resultant toxic effects.

- The partition coefficient can also be calculated. A method for logP prediction developed at Molinspiration (miLogP1.2) is based on the group contributions. Group
- 25 contributions have been obtained by fitting calculated logP with experimental logP for a training set of several thousands drug-like molecules. The method can be used by used at www.molinspiration.com/services/logp.html (QSAR 15, 403 (1996)). Many other LogP determination programs are available.

- 30 In a certain embodiment of the invention, the prodrugs of formula (I) can be used as a medicine. In another embodiment, the prodrugs of formula (I) can be used to manufacture a medicament to prevent or to treat HIV infection.

- The invention furthermore provides a method of preventing or treating HIV infection
- 35 by administering a prodrug of formula (I) by the invention. The prodrugs of formula (I) can be administered to any host, including a human, a non-human animal and mammals, in an amount effective to prevent or treat the HIV infection.

-31-

To further optimise the pharmacokinetic profile of the prodrugs of formula (I) they can be administered in conjunction with a suitable delivery vehicle (e.g., microcapsules, microspheres, biodegradable polymer films, lipid-based delivery systems such as liposomes and lipid foams, viscous instillates and absorbable mechanical barriers)
5 useful for maintaining the necessary concentrations of the prodrugs or the therapeutic compound D at the site of the disease.

The prodrug or "medicament" may be administered by any suitable method within the knowledge of the skilled man. Modes of administration known in the art for therapeutic
10 agents include parenteral, for example, intravenous (e.g. for antibody inhibitors), intraperitoneal, intramuscular, intradermal, and epidermal including subcutaneous and intradermal, oral, or application to mucosal surfaces, e.g. by intranasal administration using inhalation of aerosol suspensions, and by implanting to muscle or other tissue in the subject. Suppositories and topical, locally applied preparations are also
15 contemplated. Depending on the route and place of administration, more hydrophobic or hydrophilic peptide moieties of the prodrug can be considered.

In the present invention, the prodrugs of formula (I) are introduced in amounts sufficient to prevent, reduce or treat HIV infection.
20

The most effective mode of administration and dosage regimen for the prodrugs or the "medicament" in the methods of the present invention depend on the severity of the HIV infection, the subject's health, previous medical history, age, weight, height, sex and response to treatment and the judgment of the treating physician. Therefore, the
25 amount of prodrug to be administered, as well as the number and timing of subsequent administrations are determined by a medical professional conducting therapy based on the response of the individual subject. Initially, such parameters are readily determined by skilled practitioners using appropriate testing in animal models for safety and efficacy, and in human subjects during clinical trials of prodrug formulations. After
30 administration, the efficacy of the therapy using the prodrugs is assessed by various methods including assessment of the clinical picture.

The compounds of the present invention can thus be used in animals, preferably in mammals, and in particular in humans as pharmaceuticals per se, in mixtures with one
35 another or in the form of pharmaceutical preparations.

Furthermore, the present invention relates to pharmaceutical preparations which contain an effective dose of at least one of the prodrugs of formula (I) in addition to customary pharmaceutically innocuous excipients and auxiliaries. The pharmaceutical

-32-

preparations normally contain 0.1 to 90% by weight of the prodrug. The pharmaceutical preparations can be prepared in a manner known per se to one of skill in the art. For this purpose, at least one of a prodrug of the present invention, together with one or more solid or liquid pharmaceutical excipients and/or auxiliaries and, if
5 desired, in combination with other pharmaceutical active compounds, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human medicine or veterinary medicine.

Suitable pharmaceutical carriers for use in said pharmaceutical compositions and their
10 formulation are well known to those skilled in the art, and there is no particular restriction to their selection within the present invention. Suitable carriers or excipients known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity (such as sugars or sodium chloride) and chemical stability, buffers and
15 preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. They may also include additives such as wetting agents, dispersing agents, stickers, adhesives, emulsifying agents, solvents, coatings,
20 antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to mammals. The pharmaceutical compositions of the present invention may be prepared in any known manner, for instance by homogeneously mixing, coating and/or grinding the active ingredients, in a
25 one-step or multi-steps procedure, with the selected carrier material and, where appropriate, the other additives such as surface-active agents may also be prepared by micronisation, for instance in view to obtain them in the form of microspheres usually having a diameter of about 1 to 10 μm , namely for the manufacture of microcapsules for controlled or sustained release of the active ingredients.

30 Suitable surface-active agents to be used in the pharmaceutical compositions of the present invention are non-ionic, cationic and/or anionic materials having good emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water-soluble soaps and water-soluble synthetic surface-active agents. Suitable
35 soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids (C10-C22), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures obtainable from coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty

-33-

- 5 sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylaryl-sulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives preferably contain 8 to 22 carbon atoms. Examples of alkylarylsulphonates are the sodium, calcium or alcanolamine salts of dodecylbenzene sulphonic acid or dibutyl-naphtalenesulphonic acid or a naphtalene-sulphonic acid/formaldehyde condensation product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardiolipin, dioctanylphosphatidyl-choline, dipalmitoylphosphatidyl -choline and their mixtures.
- 20 Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkylphenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarenesulphonates and dialkylsulphosuccinates, such as polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, said derivatives preferably containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediaminopolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups. Such compounds usually contain from 1 to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol -polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/ polyethylene oxide adducts, tributyl-phenoxy polyethoxyethanol, polyethyleneglycol and octylphenoxy polyethoxyethanol.
- 35 Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants.

-34-

Suitable cationic surfactants include quaternary ammonium salts, preferably halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C₈C₂₂ alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl, oleyl and the like) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

A more detailed description of surface-active agents suitable for this purpose may be found for instance in "McCutcheon's Detergents and Emulsifiers Annual" (MC Publishing Corp., Ridgewood, New Jersey, 1981), "Tensid-Taschenbuch", 2 d ed. (Hanser Verlag, Vienna, 1981) and "Encyclopaedia of Surfactants, (Chemical Publishing Co., New York, 1981).

Additional ingredients may be included in order to control the duration of action of the active ingredient in the composition. Control release compositions may thus be achieved by selecting appropriate polymer carriers such as for example polyesters, polyamino acids, polyvinyl pyrrolidone, ethylene-vinyl acetate copolymers, methylcellulose, carboxymethylcellulose, protamine sulfate and the like. The rate of drug release and duration of action may also be controlled by incorporating the active ingredient into particles, e.g. microcapsules, of a polymeric substance such as hydrogels, polylactic acid, hydroxymethylcellulose, polyniethyl methacrylate and the other above-described polymers.

Such methods include colloid drug delivery systems like liposomes, microspheres, microemulsions, nanoparticles, nanocapsules and so on. Depending on the route of administration, the pharmaceutical composition may require protective coatings. Pharmaceutical forms suitable for injectionable use include sterile aqueous solutions or non-aqueous solutions or dispersions (suspensions, emulsions) and sterile powders for the extemporaneous preparation thereof. Typical carriers for this purpose therefore include biocompatible aqueous buffers, ethanol, glycerol, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate and the like and mixtures thereof. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases, and the like.

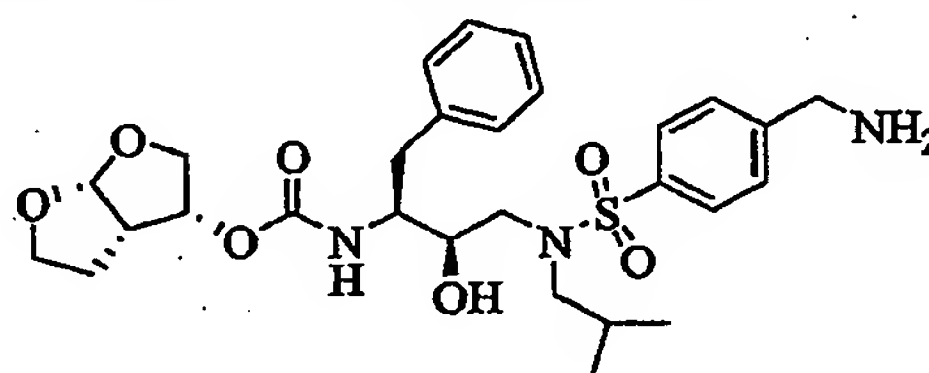
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The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

5 Experimental Part for the preparation of compounds of formula (I)

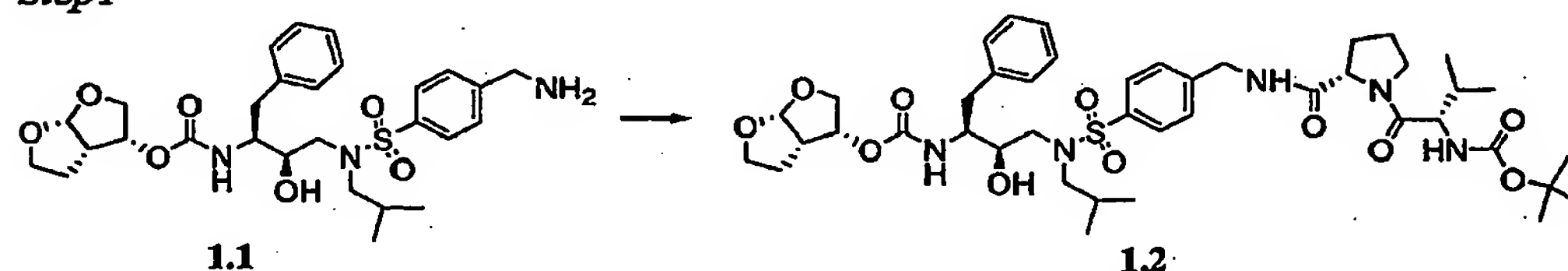
The examples describing the preparation of prodrug compounds of formula (I) will be based on the HIV protease inhibitor having the formula



hereinafter referred to as PI 1

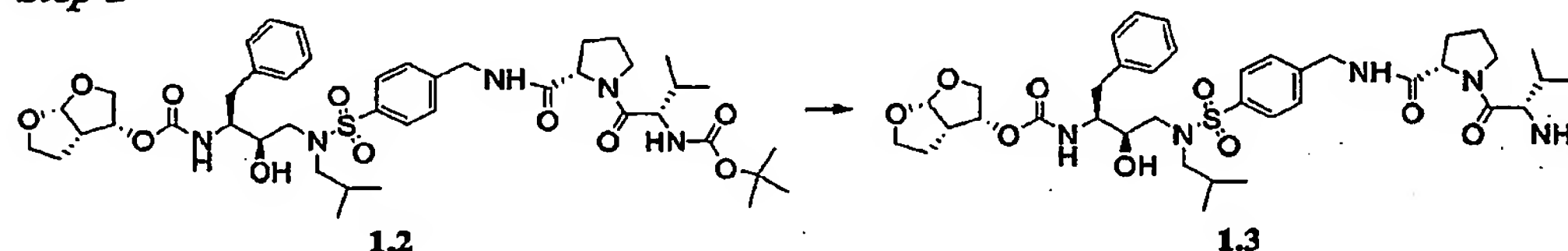
10 Example 1 : Val-Pro-PI 1

Step 1



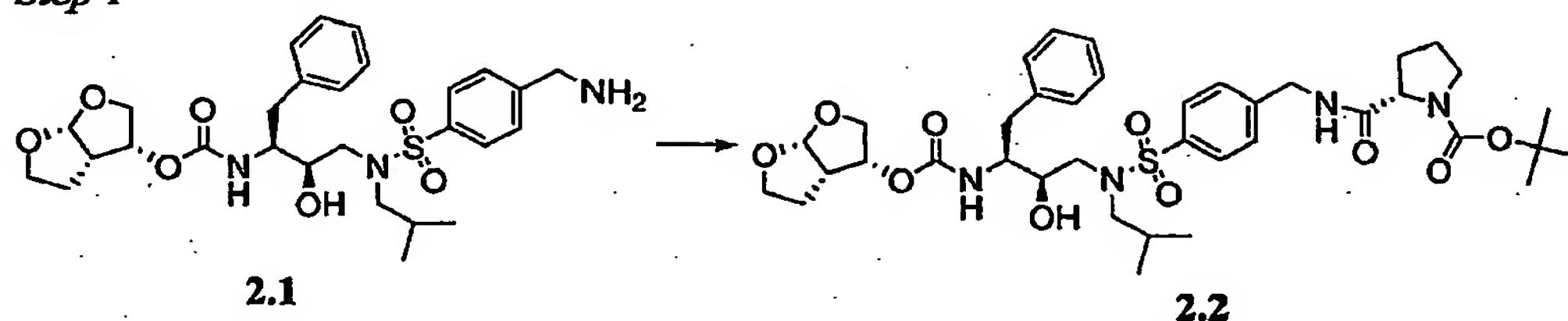
Compound 1.1 (0.95g; 1.69 mmol) and Boc-Val-Pro-OH (0.53g; 1.7 mmol) were dissolved in 10 ml *N,N*-dimethylformamide. EDCI (0.36g; 1.9 mmol) and HOAt (0.023g; 0.17 mmol) were added and stirred at room temperature for 20 hours. The reaction mixture was poured in H₂O and extracted twice with ethylacetate. The combined organic layer was washed with brine and then dried over Na₂SO₄. Solvent was removed and the obtained crude product purified by column chromatography (eluent : ethylacetate). Compound 1.2 was obtained as a white solid (yield 55%, purity 95% LC-MS).

Step 2

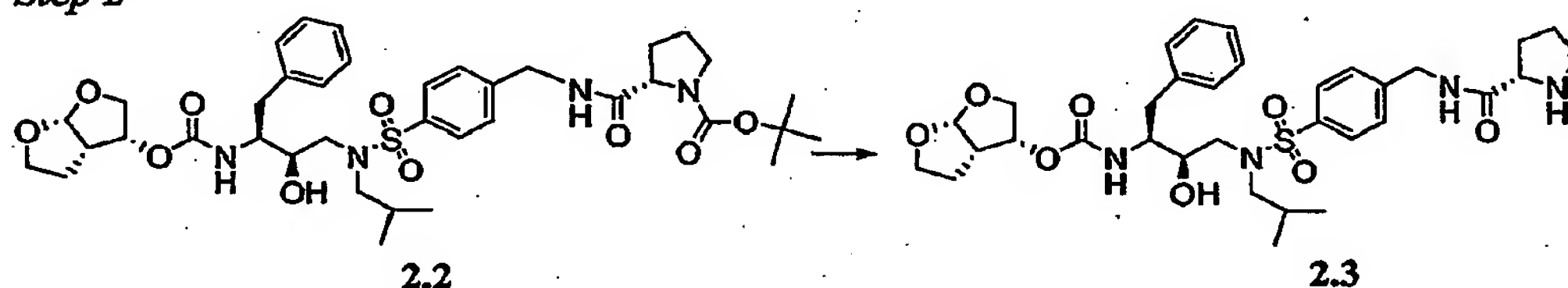


To a solution of compound 1.2 (0.77g; 0.9 mmol) in 10 ml CH₂Cl₂ was added 10 ml trifluoroacetic acid. After stirring the reaction mixture at room temperature for 3 hours, the solvent was removed. The crude mixture was purified by column chromatography yielding 0.42g of compound 1.3 (yield 61%, purity 95% LC-MS)

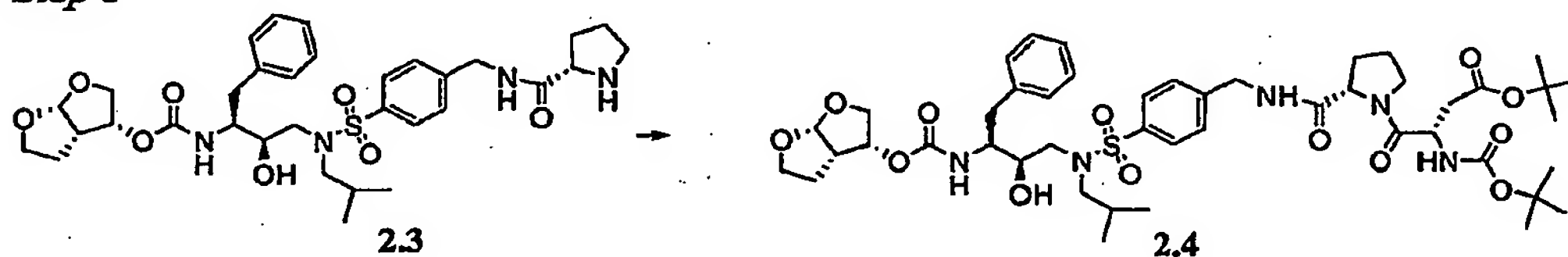
-36-

Example 2 : Asp-Pro-PI 1**Step 1**

- Compound **2.1** (3.16g; 5.63 mmol) and Boc-Pro-OH (1.33g; 6.18 mmol) were dissolved in 30 ml *N,N*-dimethylformamide. EDCI (1.18g; 6.18 mmol) and HOAt (0.077g; 0.5 mmol) were added and stirred for 36 hours. Ethylacetate and 0.1 N HCl were added and the resulting reaction mixture was extracted 3 times with ethylacetate. The combined organic layer was washed with 0.1 N HCl, H₂O, saturated NaHCO₃, water and brine. After drying over Na₂SO₄ and evaporation of the solvent a white foam (4.39g, 103%) was obtained. After trituration in diisopropylether, 3.9g of compound **2.2** was obtained (yield 93%, purity 97% LC-MS)

Step 2

- A mixture of compound **2.3** (3.7g; 4.8 mmol) and 15ml trifluoroacetic acid in 40ml CH₂Cl₂ was stirred at room temperature for 2 hours. After evaporation of solvent the crude mixture was partitioned between ethylacetate and saturated NaHCO₃. The organic layer was separated, washed with brine and dried over Na₂SO₄. Re-slurry of the crude solid in diisopropylether and filtration yielded 2.73g of compound **2.3** (yield 85%, purity >90% NMR).

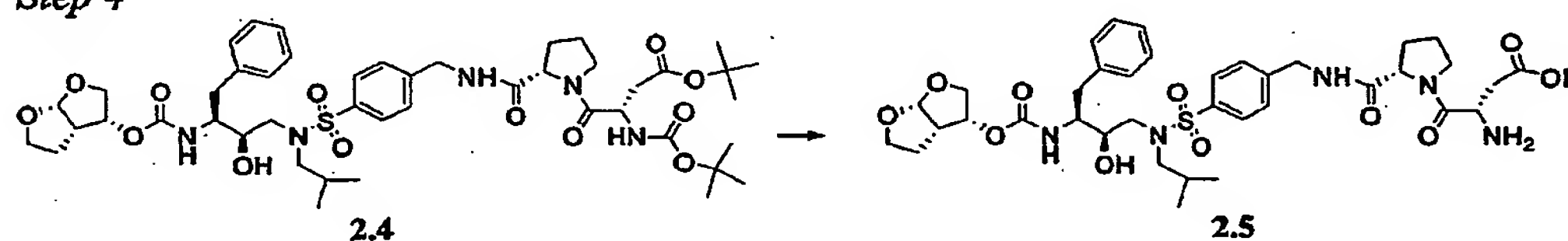
Step 3

- To a solution of compound **2.3** (1.0g; 1.5 mmol) and Boc-Asp(OtBu)-OH (0.48g; 1.7 mmol) in 30 ml *N,N*-dimethylformamide was added EDCI (0.32g; 1.7 mmol) and HOAt (0.02g; 0.15 mmol). After overnight stirring at room temperature the reaction mixture was partitioned between ethylacetate and 0.1N HCl. The H₂O-layer was

-37-

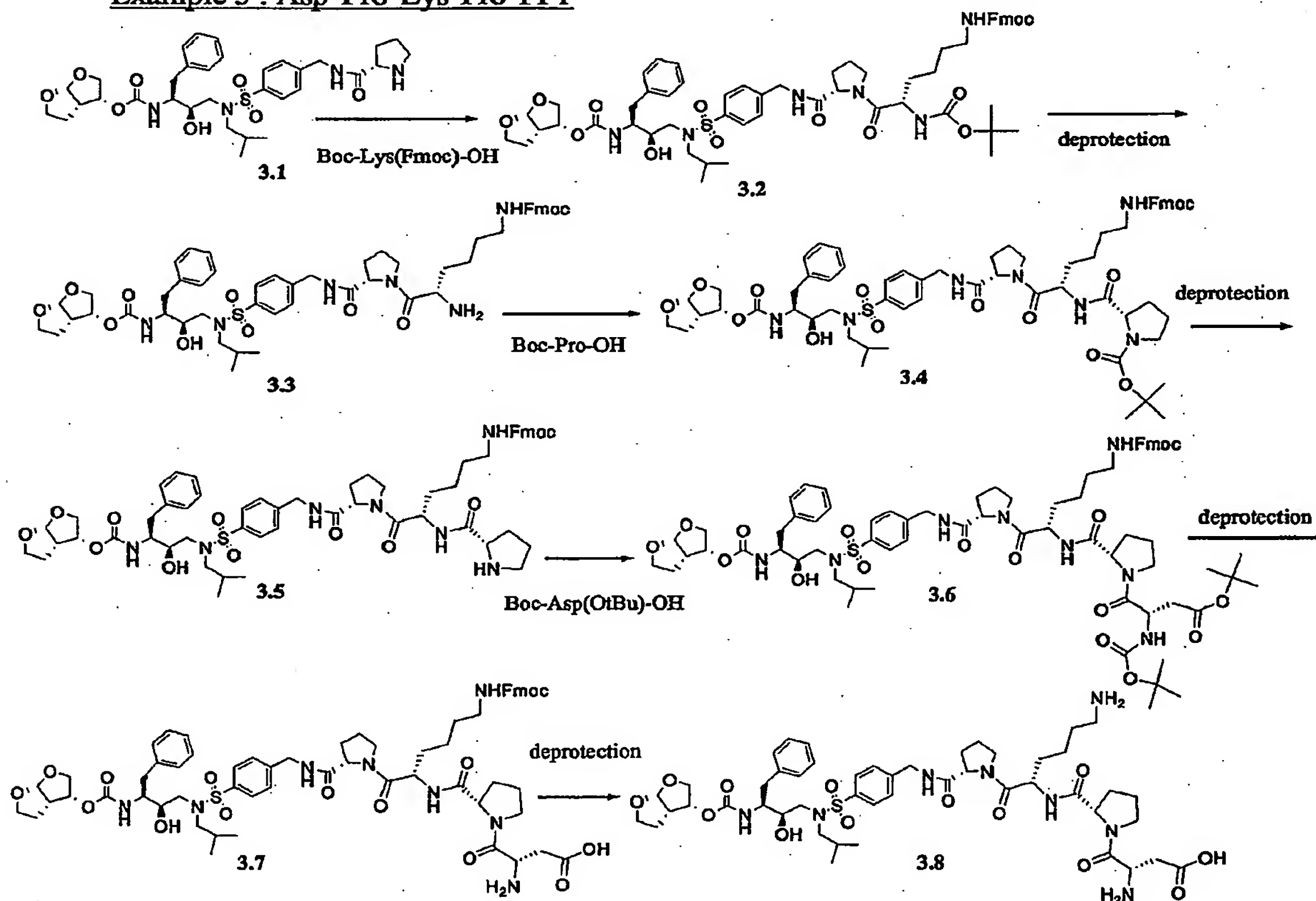
extracted 3 times and the combined organic layer was washed with 0.1N HCl, H₂O, saturated NaHCO₃ and H₂O. After drying over Na₂SO₄, the solvent was removed and the residue was triturated in diisopropylether. 1.12g of compound **2.4** was obtained (yield 79%, purity 94% LC-MS)

5

Step 4

Deprotection of compound **2.4** to **2.5** was performed in an analogously to the procedure for deprotecting compound **2.2** to compound **2.3**.

10

Example 3 : Asp-Pro-Lys-Pro-PI 1

Using analogous reaction procedures as described in examples 1 and 2, Boc-Lys(Fmoc)-OH was coupled to compound **3.1** (as prepared in example 2), yielding compound **3.2**. After Boc-deprotection, compound **3.3** was obtained. Boc-Pro-OH was then coupled to compound **3.3**, yielding compound **3.4** which was subsequently Boc-

15

-38-

deprotected thus yielding compound 3.5. Compound 3.5 was coupled with Boc-Asp(OtBu)-OH yielding compound 3.6 which was first Boc-deprotected and then Fmoc-deprotected using dimethylamine in tetrahydrofuran, thus yielding compound 3.8 corresponding to Asp-Pro-Lys-Pro-PI 1.

5

Example 4 : Conversion of Val-Pro-PI 1 to PI 1 by purified CD26, human and bovine serum

The dipeptide (Val-Pro) derivative of PI 1 (Val-Pro-PI 1) was exposed to purified CD26 (Fig. 10), and 10% or 2% human or bovine serum, diluted in PBS (phosphate-buffered saline) (Fig. 10 and 11). Val-Pro-PI 1 was efficiently converted to PI 1 in all conditions tested. Within 60 minutes, Val-Pro-PI 1 was completely converted to PI 1 by purified CD26. Ten percent BS or HS converted 40 to 70% of Val-Pro-PI 1 to PI 1 in one hour (Fig. 10). Two percent BS and HS converted Val-Pro-PI 1 to PI 1 by 8% and 25%, respectively. After 4 hrs, 35% and 95% of compound was hydrolyzed by BS and HS, respectively (Fig. 11).

15

In the presence of 50 μ M GP-pNA (glycylprolyl-para-nitroanilide), 100 μ M Val-Pro-PI 1 efficiently competed with the substrate for CD26 (Fig. 12). Also 20 μ M Val-Pro-PI 1 could inhibit the release of pNA from GP-pNA, presumably by competitive inhibition of the CD26-catalysed reaction. Conversion of GP-pNA to pNA by two percent BS in PBS was even more efficiently inhibited by Val-Pro-PI 1 than purified CD26 (Fig. 13). Also HS (2% in PBS)-catalysed GP-pNA conversion to pNA was competitively inhibited by Val-Pro-PI 1 (Fig. 14).

20

Example 5: Separation Val-Pro-PI 1 and PI 1 compounds

Compounds were separated on a Reverse Phase RP-8 (Merck) using a gradient with buffer A (50 mM NaH_2PO_4 + 5 mM heptane sulfonic acid pH 3.2) and buffer B (acetonitrile).

25

0 \rightarrow 2 min: 2% buffer B; 2 \rightarrow 8 min: 20% buffer B; 8 \rightarrow 10 min: 25% buffer B; 10 \rightarrow 12 min: 35% buffer B; 12 \rightarrow 30 min: 50% buffer B; 30 \rightarrow 35 min: 50% buffer B; 35 \rightarrow 40 min: 2% buffer B; 40 \rightarrow 45 min: 2% buffer B. Rt values of Val-Pro-PI 1 and PI 1 were 18.7 and 17.7 min, respectively.

30

Example 6 : pharmaceutical compositions

Capsules

A compound of formula (I), is dissolved in organic solvent such as ethanol, methanol or methylene chloride, preferably, a mixture of ethanol and methylene chloride. Polymers such as polyvinylpyrrolidone copolymer with vinyl acetate (PVP-VA) or hydroxypropylmethylcellulose (HPMC), typically 5 mPa.s, are dissolved in organic

35

-39-

solvents such as ethanol, methanol methylene chloride. Suitably the polymer is dissolved in ethanol. The polymer and compound solutions are mixed and subsequently spray dried. The ratio of compound/polymer was selected from 1/1 to 1/6. Intermediate ranges are 1/1.5 and 1/3. A suitable ratio is 1/6. The spray-dried powder, a solid dispersion, is subsequently filled in capsules for administration. The drug load in one capsule ranges between 50 and 100 mg depending on the capsule size used.

Film-coated Tablets

Preparation of Tablet Core

10 A mixture of 100 g of a compound of formula (I), 570 g lactose and 200 g starch is mixed well and thereafter humidified with a solution of 5 g sodium dodecyl sulfate and 10 g polyvinylpyrrolidone in about 200 ml of water. The wet powder mixture is sieved, dried and sieved again. Then there was added 100 g microcrystalline cellulose and 15 g hydrogenated vegetable oil. The whole is mixed well and compressed into tablets, giving 10.000 tablets, each comprising 10 mg of the prodrug of formula (I).

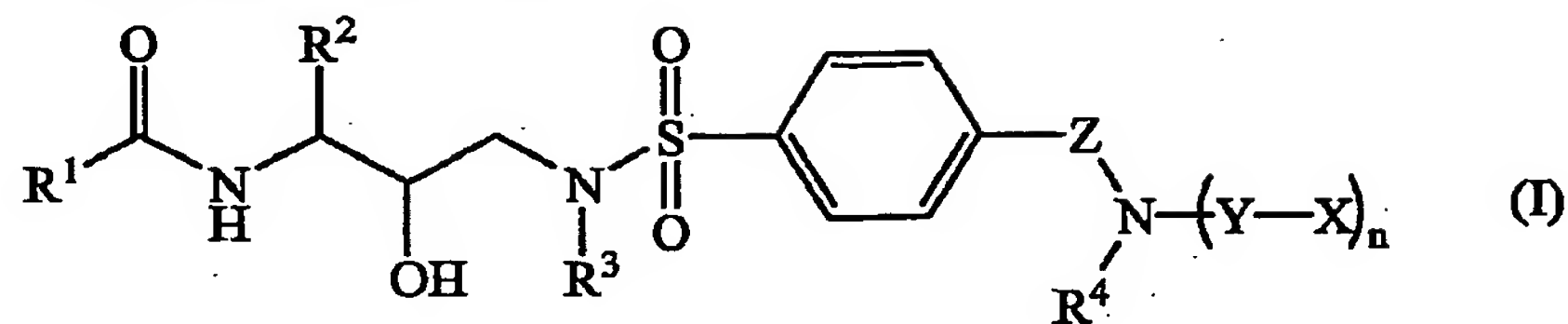
Coating

To a solution of 10 g methylcellulose in 75 ml of denaturated ethanol there is added a solution of 5 g of ethylcellulose in 150 ml of dichloromethane. Then there are added 75 ml of dichloromethane and 2.5 ml 1,2,3-propanetriol. 10 g of polyethylene glycol is molten and dissolved in 75 ml of dichloromethane. The latter solution is added to the former and then there are added 2.5 g of magnesium octadecanoate, 5 g of polyvinylpyrrolidone and 30 ml of concentrated color suspension and the whole is homogenated. The tablet cores are coated with the thus obtained mixture in a coating apparatus.

25

CLAIMS

1. A prodrug having the formula



- 5 the stereoisomeric form or salt thereof, wherein
 n is 1, 2, 3, 4 or 5;
 Y is proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid
 (thiopline), dehydroproline, pipecolic acid (L-homoproline),
 azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine,
 10 leucine, isoleucine and threonine;
 X is selected from any amino acid in the D- or L-configuration;
 X and Y in each repeat of [Y-X] are chosen independently from one another and
 independently from other repeats;
 Z is a direct bond or a bivalent straight or branched saturated hydrocarbon group
 15 having from 1 to 4 carbon atoms;
 R¹ is an aryl, heteroaryl, aryloxy, heteroaryloxy, aryloxyC₁₋₄alkyl,
 heterocycloalkyloxy, heterocycloalkylC₁₋₄alkyloxy, heteroaryloxyC₁₋₄alkyl,
 heteroarylC₁₋₄alkyloxy;
 R² is arylC₁₋₄alkyl;
 20 R³ is C₁₋₁₀alkyl, C₂₋₆alkenyl or C₃₋₇cycloalkylC₁₋₄alkyl;
 R⁴ is hydrogen or C₁₋₄alkyl;
 aryl, when used alone or in combination with another group, means phenyl
 optionally substituted with one or more substituents each individually selected
 from the group consisting of C₁₋₄alkyl, hydroxy, C₁₋₄alkyloxy, nitro, cyano,
 25 halo, amino, mono- or di(C₁₋₄alkyl)amino and amido;
 heteroaryl, when used alone or in combination with another group, means a
 monocyclic or bicyclic aromatic heterocycle having one or more oxygen,
 sulphur or nitrogen heteroatoms, which aromatic heterocycle may optionally
 be substituted on one or more carbon atoms with a substituent selected from
 30 the group consisting of C₁₋₄alkyl, C₁₋₄alkyloxy, amino, hydroxy, aryl, amido,
 mono- or di(C₁₋₄alkyl)amino, halo, nitro, heterocycloalkyl and C₁₋₄
 alkyloxycarbonyl, and which aromatic heterocycle may also be optionally
 substituted on a secondary nitrogen atom by C₁₋₄alkyl or arylC₁₋₄alkyl;

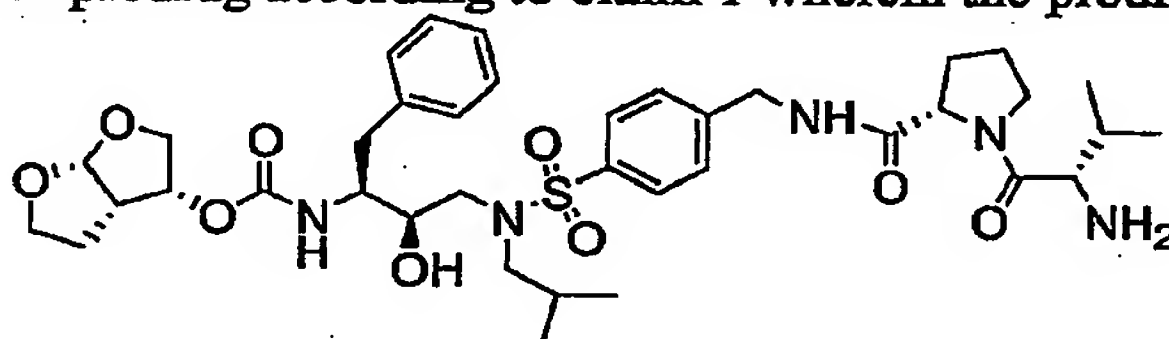
-41-

- heterocycloalkyl, when used alone or in combination with another group, means a saturated or partially unsaturated monocyclic or bicyclic heterocycle having one or more oxygen, sulphur or nitrogen heteroatoms, which heterocycle may optionally be substituted on one or more carbon atoms with a substituent selected from the group consisting of C₁₋₄alkyl, C₁₋₄alkyloxy, hydroxy, halo and oxo, and which heterocycle may also be optionally substituted on a secondary nitrogen atom by C₁₋₄alkyl or arylC₁₋₄alkyl.
- 5
2. A prodrug as claimed in claim 1 wherein each X independently is selected from a naturally occurring amino acid.
- 10
3. A prodrug as claimed in claim 1 or 2 wherein n is 1, 2 or 3.
4. A prodrug as claimed in any one of claims 1 to 3 wherein n is 2 or 3 and wherein at least one X is an hydrophobic or aromatic amino acid.
5. A prodrug as claimed in any one of claims 1 to 4 wherein n is 2 or 3 and wherein at least one X is an neutral or acidic amino acid.
- 15
6. A prodrug as claimed in any one of claims 1 to 5 wherein n is 2 or 3 and wherein at least one X is a basic amino acid.
7. A prodrug as claimed in any one of claims 1 to 6 wherein -(Y-X)_n comprises amino-terminally X-Pro, X-Ala, X-Gly, X-Ser, X-Val, or X-Leu.
8. A prodrug as claimed in any one of claims 1 to 7 wherein -(Y-X)_n comprises amino-terminally X-proline or X-alanine.
- 20
9. A prodrug as claimed in any one of claims 1 to 8 wherein each Y independently is proline, alanine, glycine, serine, valine or leucine.
10. A prodrug as claimed in any one of claims 1 to 9 wherein each Y independently is proline or hydroxyproline or dihydroxyproline or alanine.
- 25
11. A prodrug as claimed in any one of claims 1 to 10 wherein each Y independently is proline or alanine.
12. A prodrug as claimed in any one of claims 1 to 11 wherein -(Y-X)_n is -(Y-X)_{1or2}-Y-Val.
13. A prodrug as claimed in any one of claims 1 to 12 wherein -(Y-X)_n is -(Y-X)_{1or2}-Pro-Val.
- 30
14. A prodrug as claimed in any one of claims 1 to 13 wherein the (Y-X)_n oligopeptide is built up with (Y-X) repeats selected from the group consisting of Pro-Val,

-42-

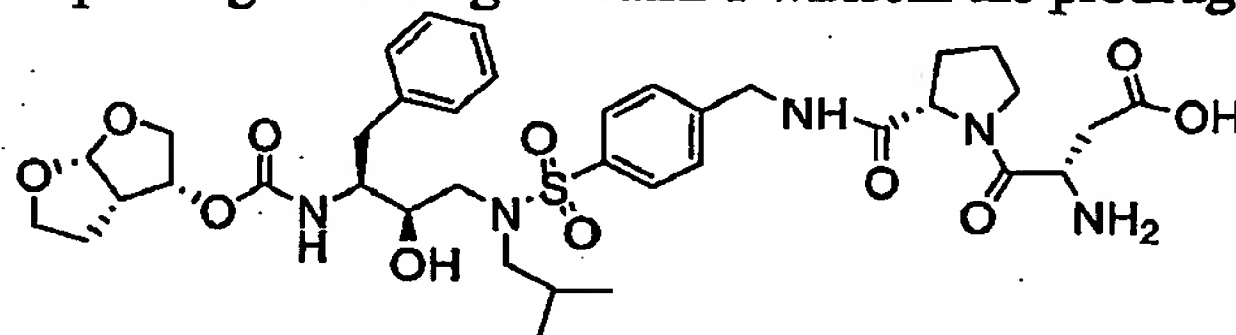
Pro-Asp, Pro-Ser, Pro-Lys, Pro-Arg, Pro-His, Pro-Phe, Pro-Ile, Pro-Leu, Ala-Val, Ala-Asp, Ala-Ser, Ala-Lys, Ala-Arg, Ala-His, Ala-Phe, Ala-Ile and Ala-Leu.

15. A prodrug as claimed in any one of claims 1 to 14 wherein R^1 is heterocycloalkyloxy, heteroaryl, heteroaryl C_{1-4} alkyloxy, aryl or aryloxy C_{1-4} alkyl.
- 5 16. A prodrug as claimed in any one of claims 1 to 15 wherein R^1 is hexahydrofuro[2,3-b]furan-3-yl-oxy, tetrahydrofuran-3-yl-oxy, quinolin-2-yl, thiazol-5-ylmethyloxy, 3-hydroxy-2-methyl-1-phenyl, 2,6-dimethylphenoxymethyl.
17. A prodrug as claimed in any one of claims 1 to 16 wherein R^1 is hexahydrofuro[2,3-b]furan-3-yl-oxy, tetrahydrofuran-3-yl-oxy, quinolin-2-yl, 10 thiazol-5-ylmethyloxy, 3-hydroxy-2-methyl-1-phenyl, 2,6-dimethylphenoxymethyl.
18. A prodrug as claimed in any one of claims 1 to 17 wherein R^1 is (3R, 3aS, 6aR)-hexahydrofuro[2,3-b]furan-3-yl-oxy.
19. A prodrug as claimed in any one of claims 1 to 18 wherein R^2 is phenylmethyl; R^3 is isobutyl and R^4 is hydrogen.
- 15 20. A prodrug as claimed in any one of claims 1 to 19 wherein Z is methylene.
21. A prodrug according to claim 1 wherein the prodrug is



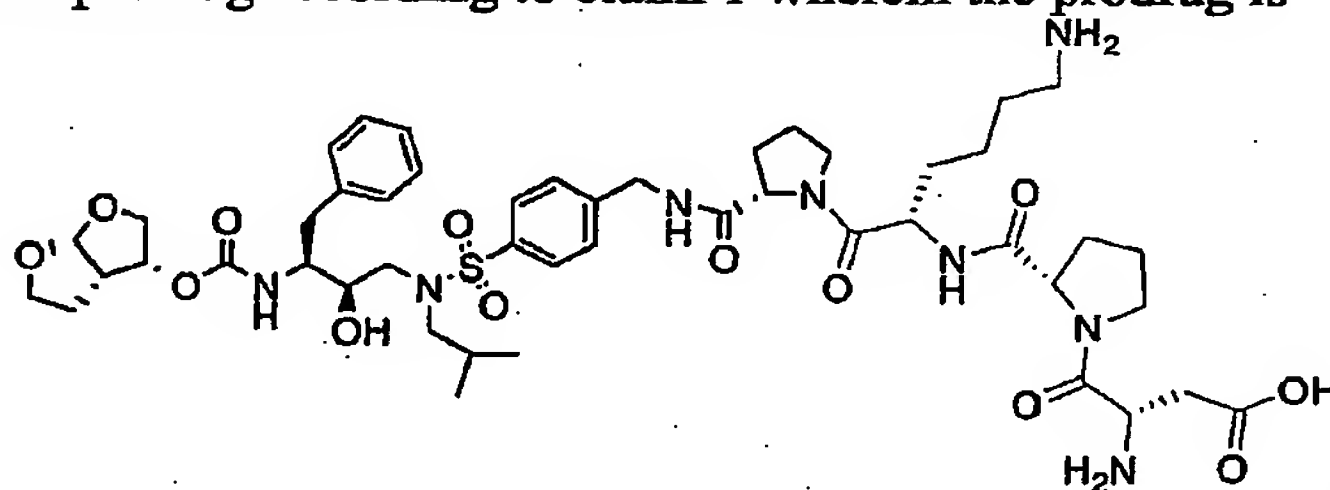
or a salt thereof.

22. A prodrug according to claim 1 wherein the prodrug is



or a salt thereof.

- 20 23. A prodrug according to claim 1 wherein the prodrug is



or a salt thereof.

24. A prodrug according to any one of claims 1 to 23 for use as a medicine.

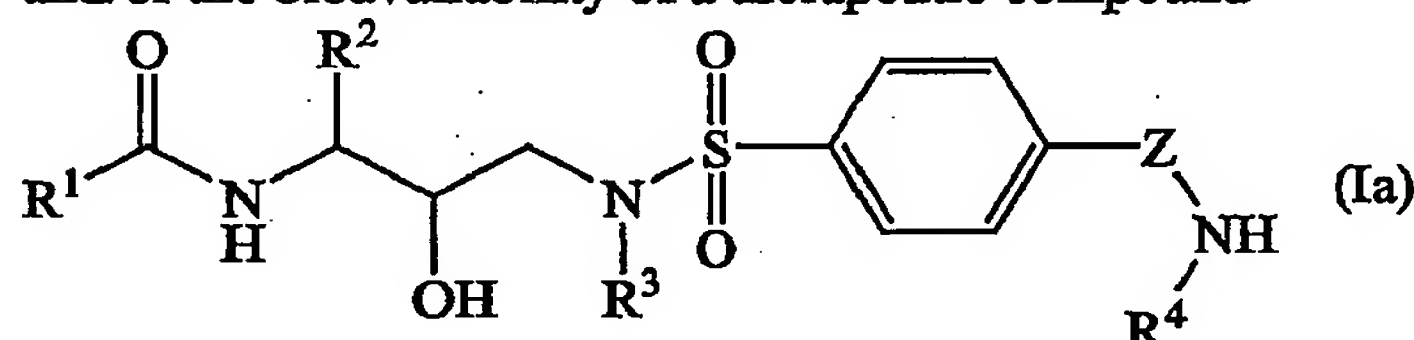
-43-

25. Use of a prodrug according to any one of claims 1 to 23 for the manufacture of a medicament useful for preventing or treating HIV infection.

26. A method of preventing or treating HIV infection by administering to any host, including a human, a non-human animal and mammals, a prodrug according to any one of claims 1 to 23 in an amount effective to prevent or treat the HIV infection.

27. A pharmaceutical preparation which contains an effective dose of at least one of the prodrugs as claimed in any one of claims 1 to 23 in addition to customary pharmaceutically innocuous excipients and auxiliaries.

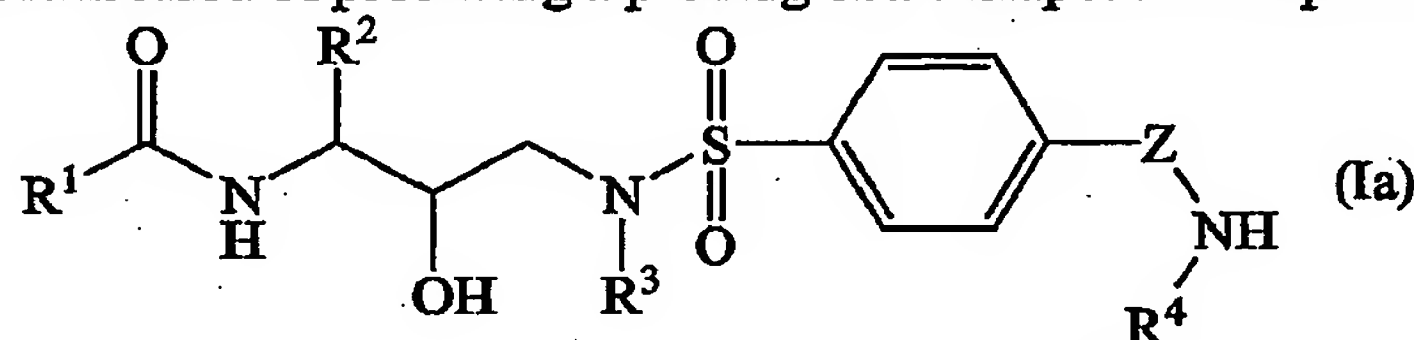
28. A method for modulating the water solubility, modulating plasma protein binding and/or the bioavailability of a therapeutic compound



by coupling a peptide of formula H-(X-Y)_n to said prodrug wherein n, X, Y, R¹, R², R³, R⁴ and Z are as defined in any one of claims 1 to 23 and wherein the resulting conjugate is cleavable by a dipeptidyl-peptidase.

29. A method according to claim 28 wherein the dipeptidyl-peptidase is CD26.

30. A method of producing a prodrug of a therapeutic compound



wherein the prodrug is cleavable by a dipeptidyl-peptidase, the method comprising the step of linking a therapeutic compound and a peptide of formula H-(X-Y)_n wherein n, X, Y, R¹, R², R³, R⁴ and Z are as defined in any one of claims 1 to 20 and wherein the resulting conjugate is cleavable by a dipeptidyl-peptidase.

31. A method according to claim 30 wherein the dipeptidyl-peptidase is CD26.

1/5

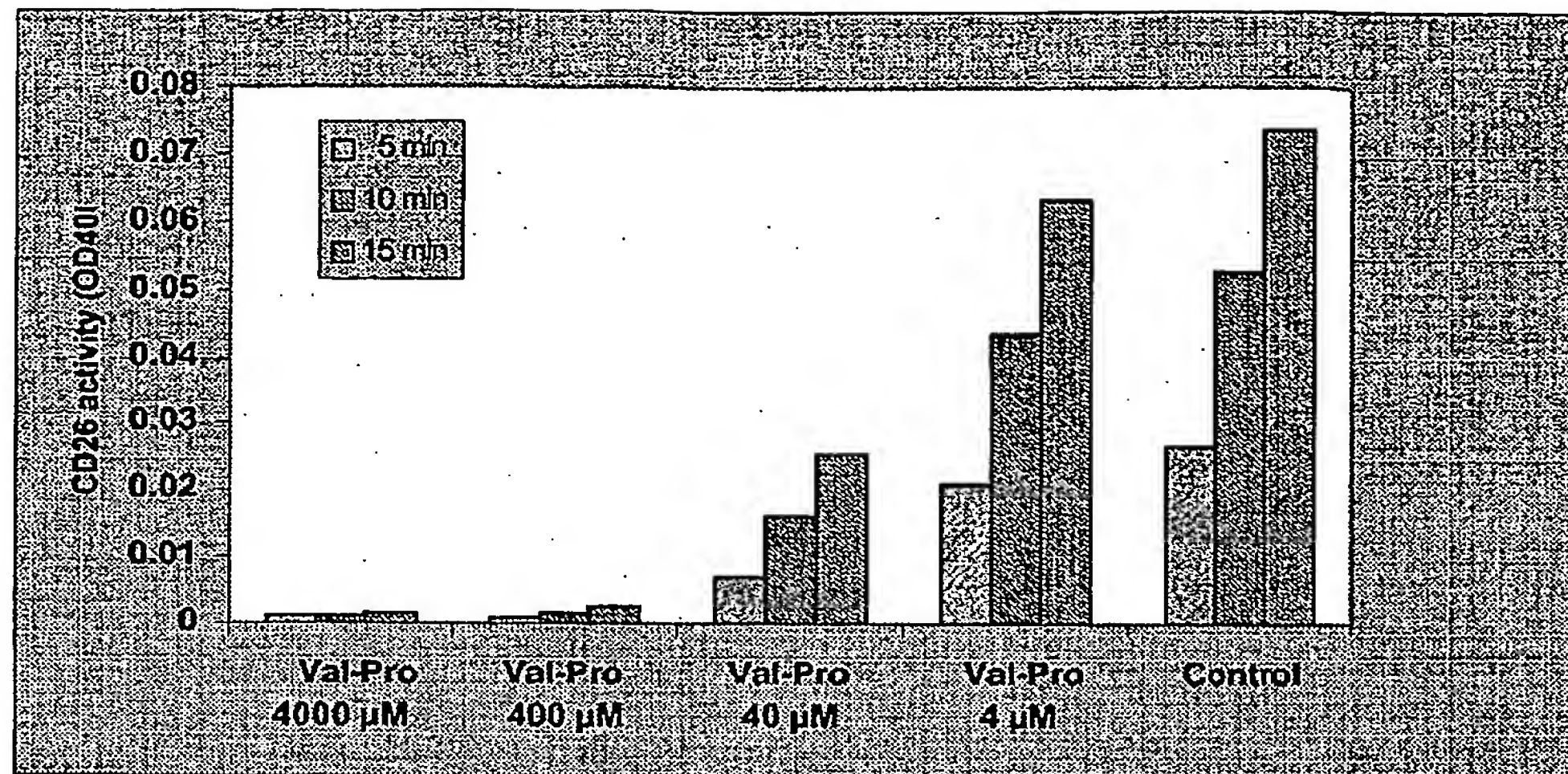


Figure 1

2/5

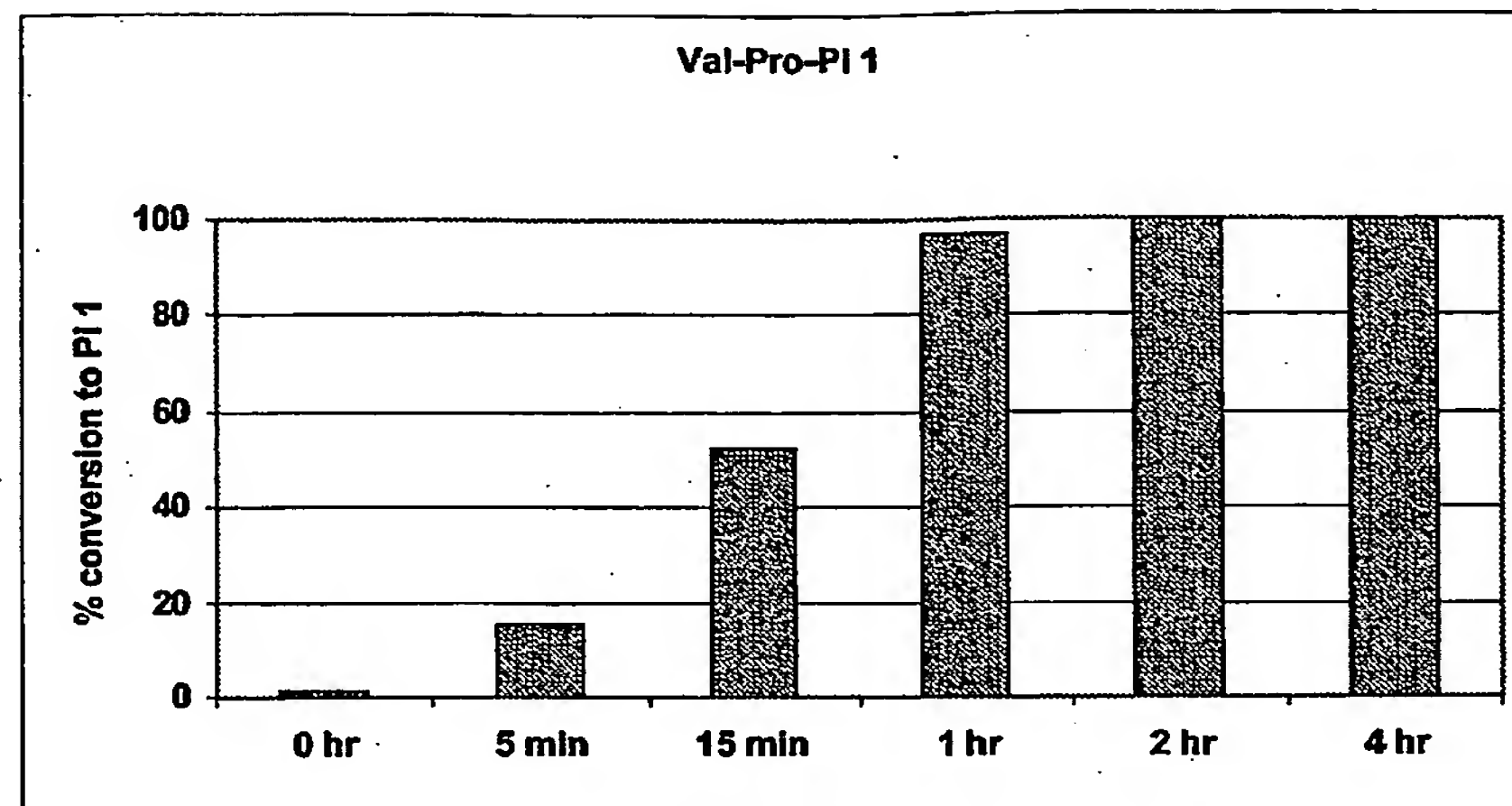


Figure 2a

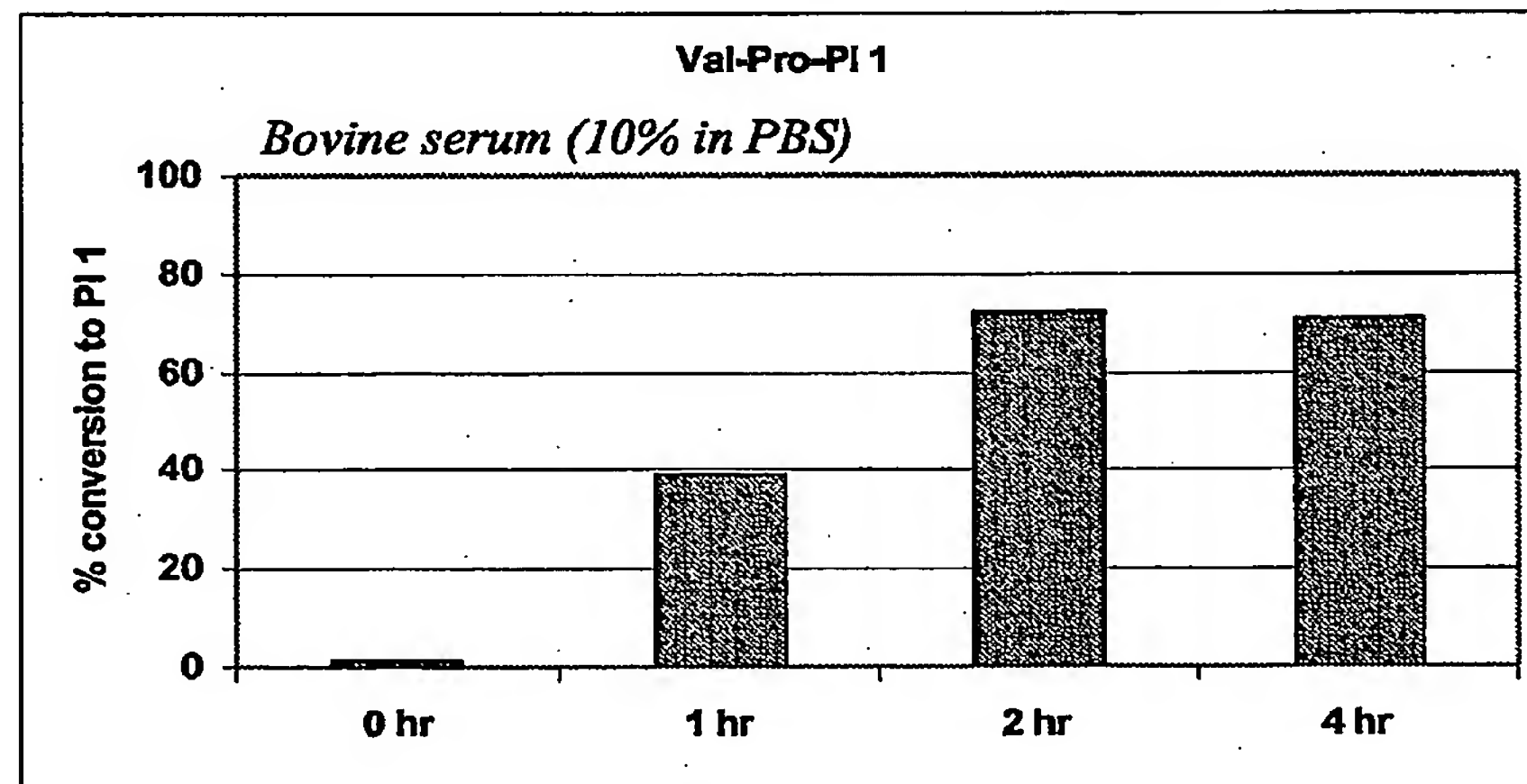


Figure 2b

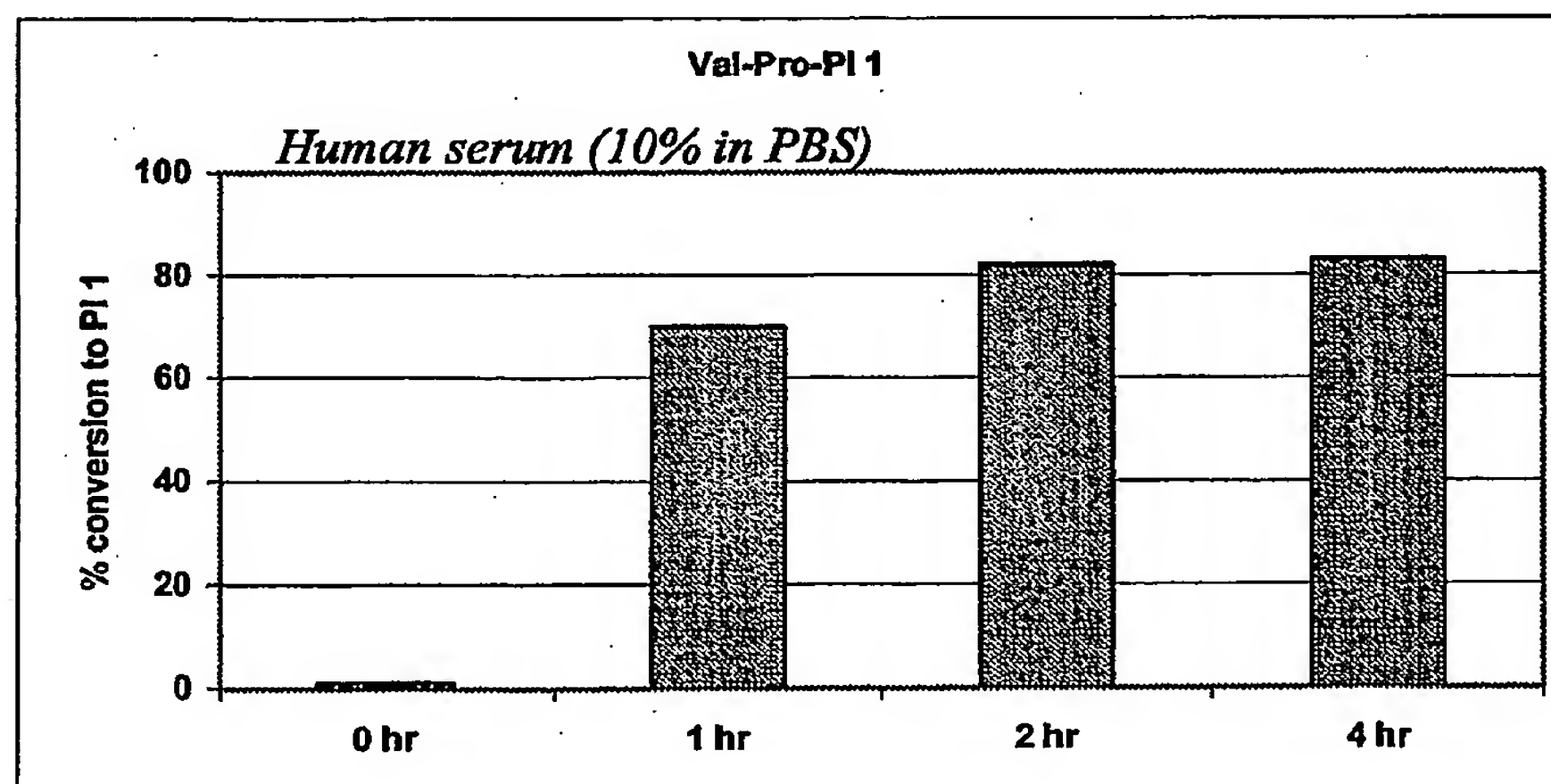


Figure 2c

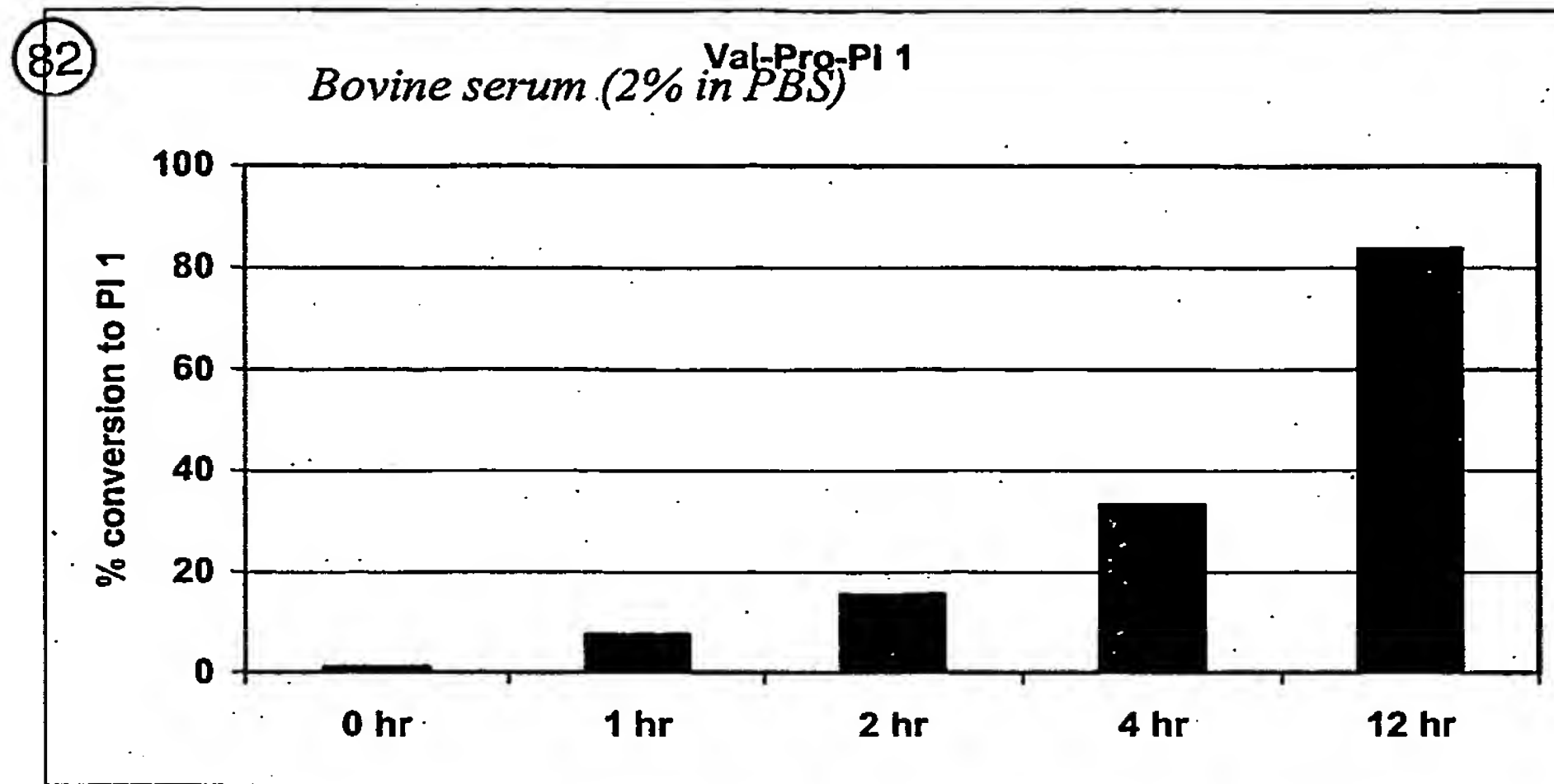


Figure 3a

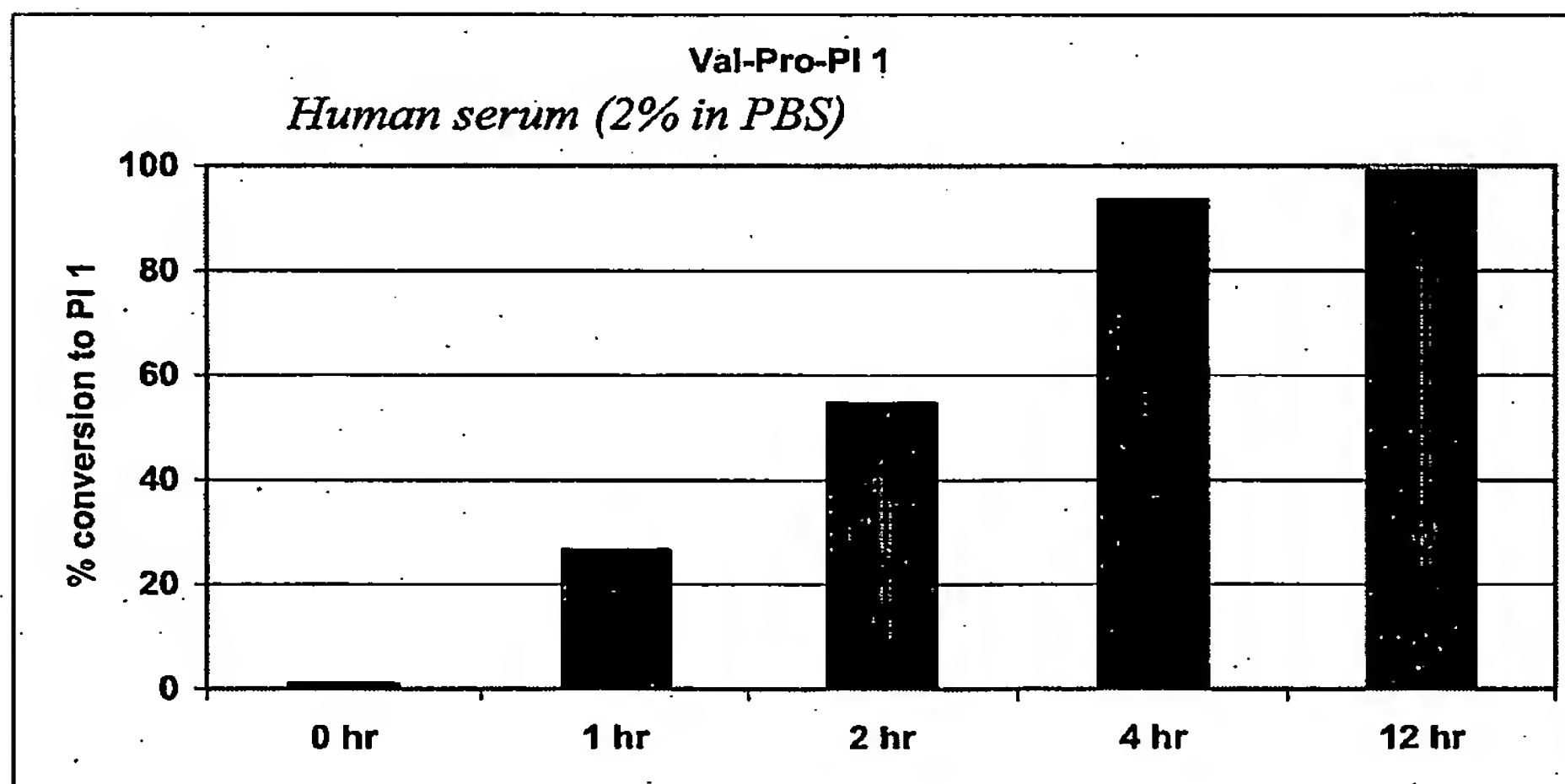


Figure 3b

4/5

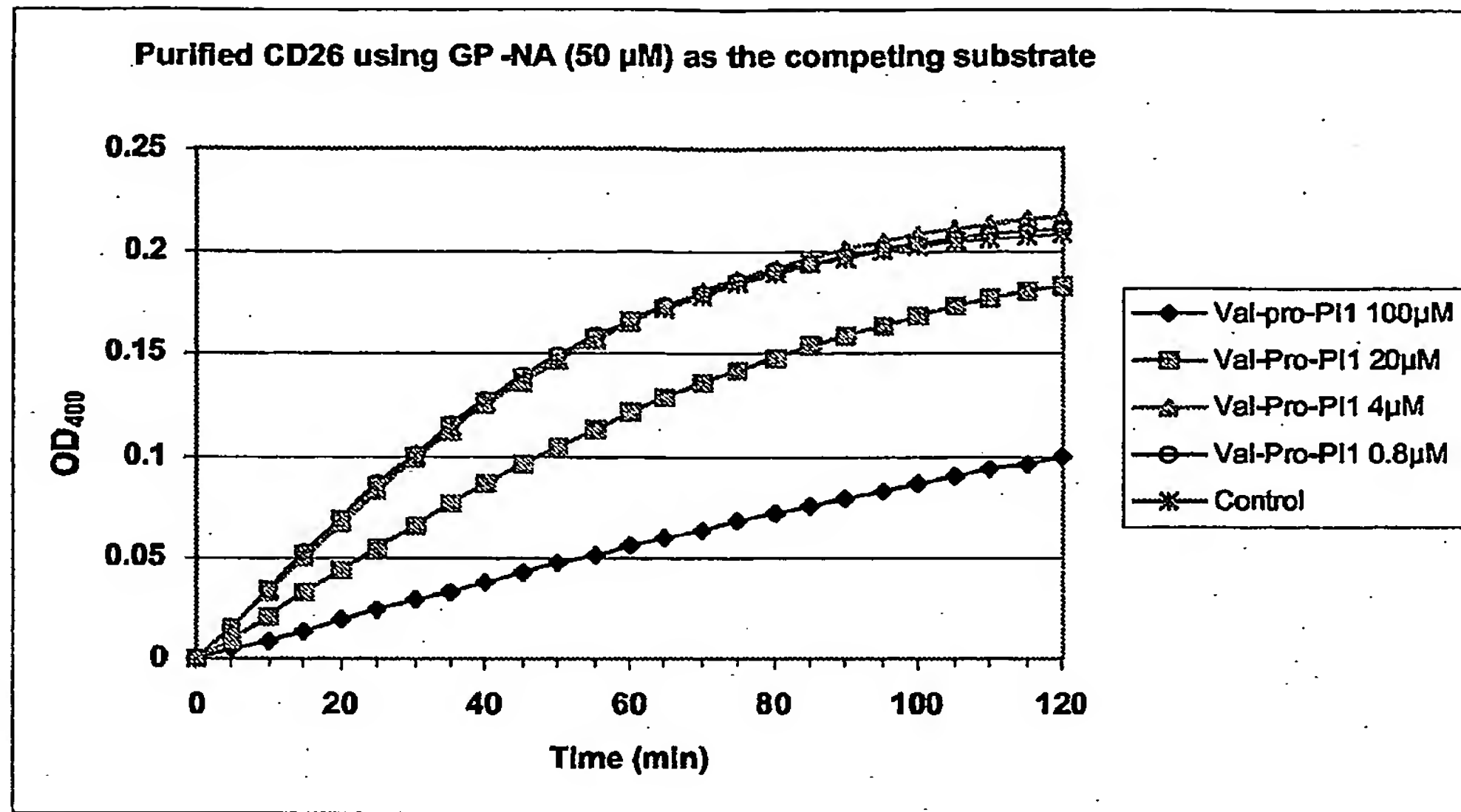


Figure 4

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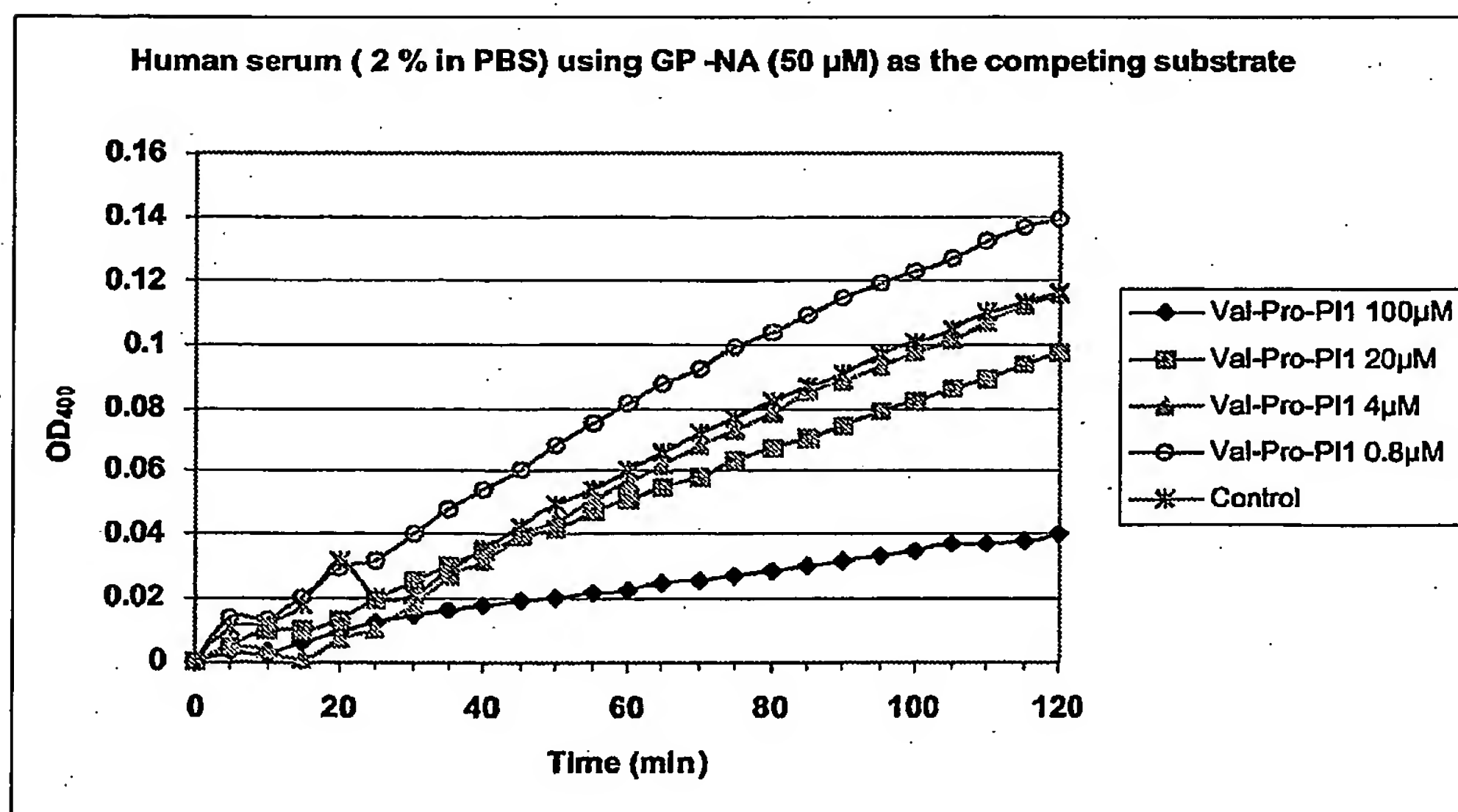


Figure 5

5/5

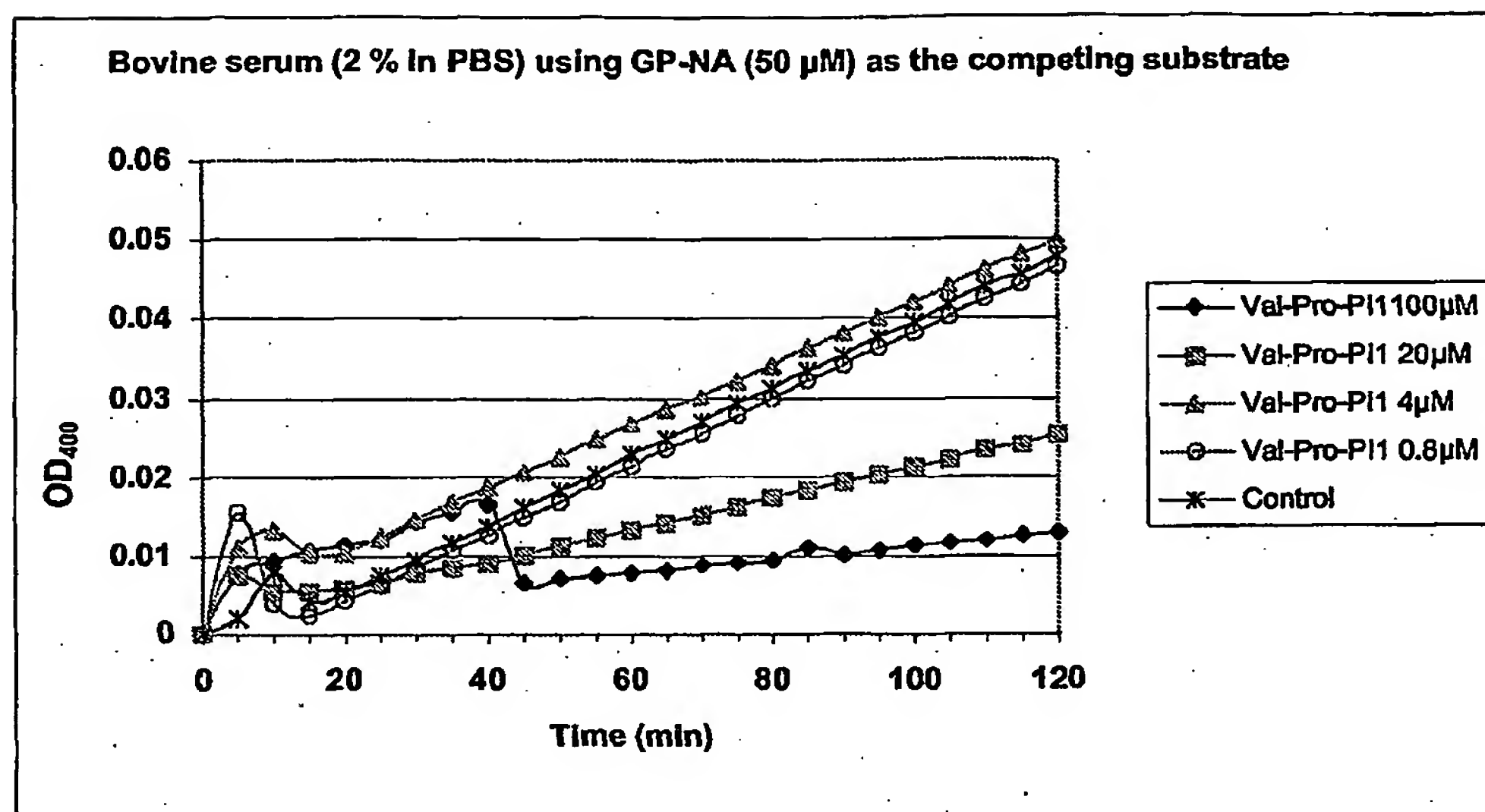


Figure 6

10/555712

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SEQUENCE LISTING

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<151> 2003-05-08

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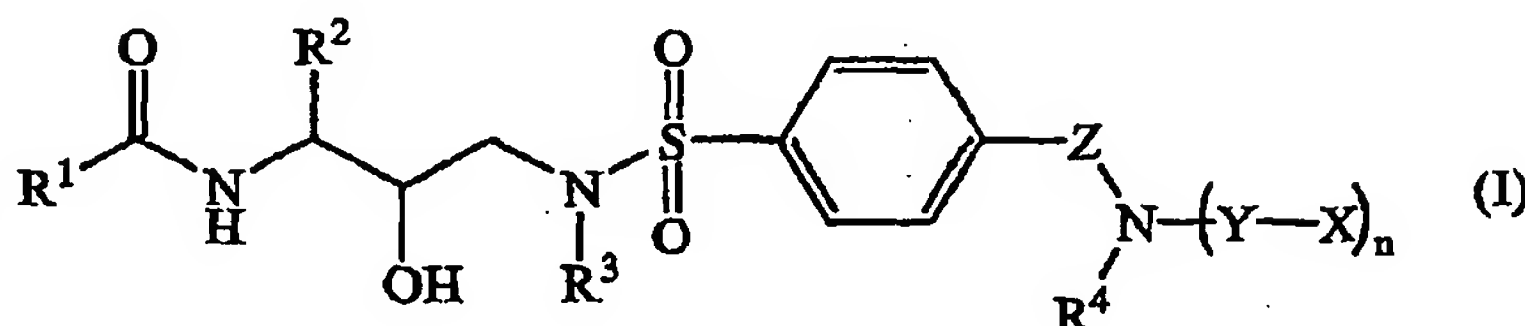
- as to applicant's entitlement to apply for and be granted
a patent (Rule 4.17(ii)) for the following designations AE,
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IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii)) for all designations
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Published:

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[Continued on next page]

(54) Title: HIV PRODRUGS CLEAVABLE BY CD26



(57) Abstract: The present invention
provides new prodrugs which are
conjugates of a therapeutic compound
and a peptide wherein the conjugate
is cleavable by dipeptidyl-peptidases,
more preferably by CD26, also known as
DPPIV (dipeptidyl aminodiesterase IV).

The present prodrugs have the formula
(I), the stereoisomeric forms and salts thereof, wherein n is 1 to 5; Y is proline, alanine, hydroxyproline, dihydroxyproline, thiazolidinecarboxylic acid (thioprolin), dehydroproline, pipercolic acid (L-homoprolin), azetidinecarboxylic acid, aziridinecarboxylic acid, glycine, serine, valine, leucine, isoleucine and threonine; X is selected from any amino acid in the D- or L-configuration; X and Y in each repeat of [Y-X] are chosen independently from one another and independently from other repeats; Z is a direct bond or a bivalent straight or branched saturated hydrocarbon group having from 1 to 4 carbon atoms; R¹ is an aryl, heteroaryl, aryloxy; heteroaryloxy, aryloxyC₁₋₄alkyl, heterocycloalkyloxy, heterocycloalkylC₁₋₄alkoxy, heteroaryloxyC₁₋₄alkyl, heteroarylC₁₋₄alkyloxy; R² is arylC₁₋₄alkyl; R³ is C₁₋₁₀alkyl, C₂₋₆alkenyl or C₃₋₇cycloalkylC₁₋₄alkyl; R⁴ is hydrogen or C₁₋₄alkyl. The present invention furthermore provides the use of said prodrugs as medicines as well as a method of producing said prodrugs.



— with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/45117 A (PROTOTEK INC ; SMITH ROBERT E (US)) 4 December 1997 (1997-12-04) the whole document	1-31
A	WO 00/71571 A (BOEHRINGER SOHN INGELHEIM ; GARIN CHESA PILAR (DE); RETTIG WOLFGANG J) 30 November 2000 (2000-11-30) the whole document	
	----- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- "&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/050753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BALAJTHY Z ET AL: "SYNTHESIS AND FUNCTIONAL EVALUATION OF A PEPTIDE DERIVATIVE OF 1-BETA-D-ARABINOFURANOSYLCYTOSINE" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 35, no. 18, 1992, pages 3344-3349, XP002939110 ISSN: 0022-2623 the whole document	
A	TROUET A ET AL: "Extracellularly tumor - activated prodrugs fo the selective chemotherapy of cancer: application to doxorubicin and preliminary in vitro and in vivo studies" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 61, no. 7, 1 April 2001 (2001-04-01), pages 2843-2846, XP002204750 ISSN: 0008-5472 the whole document	
A	US 5 962 216 A (BAURAIN ROGER ET AL) 5 October 1999 (1999-10-05) the whole document	
A	MASQUELIER M ET AL: "AMINO ACID AND DIPEPTIDE DERIVATIVES OF DAUNORUBICIN. 1. SYNTHESIS,PHYSICOCHEMICAL PROPERTIES, AND LYSOSOMAL DIGESTION" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 23, no. 11, 1980, pages 1166-1170, XP000914522 ISSN: 0022-2623 the whole document	
A	WO 99/67278 A (MANHART SUSANNE ; DEMUTH HANS ULRICH (DE); HOFFMANN TORSTEN (DE); PROB) 29 December 1999 (1999-12-29) the whole document	
A	ANAND BANMEET ET AL: "Novel dipeptide prodrugs of acyclovir for ocular herpes infections: Bioreversion, antiviral activity and transport across rabbit cornea." CURRENT EYE RESEARCH. 2003 MAR-APR, vol. 26, no. 3-4, March 2003 (2003-03), pages 151-163, XP009036464 ISSN: 0271-3683 the whole document	

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/050753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AUGUSTYNS K ET AL: "THE UNIQUE PROPERTIES OF DIPEPTIDYL-PEPTIDASE IV (DPP IV/CD26) AND THE THERAPEUTIC POTENTIAL OF DPP IV INHIBITORS" CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS BV, BE, vol. 6, no. 4, 1999, pages 311-327, XP000870290 ISSN: 0929-8673 the whole document	
A	YARON A ET AL: "PROLINE-DEPENDENT STRUCTURAL AND BIOLOGICAL PROPERTIES OF PEPTIDES AND PROTEINS" CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, CRC PRESS, BOCA RATON, FL, US, vol. 28, no. 1, 1993, pages 31-81, XP000925565 ISSN: 1040-9238 the whole document	
A	DE MEESTER I ET AL: "CD26, let it cut or cut it down" IMMUNOLOGY TODAY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 20, no. 8, 1 August 1999 (1999-08-01), pages 367-375, XP004177365 ISSN: 0167-5699 cited in the application the whole document	
P,X	WO 03/048190 A (MITRA ASHIM K ; UNIV MISSOURI (US)) 12 June 2003 (2003-06-12) the whole document	1,11-13, 25,26, 31,32, 43,44, 50,51, 54,55
Y	WO 99/67254 A (UNIV ILLINOIS ; US HEALTH (US); ERICKSON JOHN W (US); GHOSH ARUN K (US) 29 December 1999 (1999-12-29) figure 5a	1-31
Y	BOONACKER EMIL ET AL: "The multifunctional or moonlighting protein CD26/DPPIV." EUROPEAN JOURNAL OF CELL BIOLOGY, vol. 82, no. 2, February 2003 (2003-02), pages 53-73, XP002302224 ISSN: 0171-9335 the whole document	1-31
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/050753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>OHTSUKI TAKASHI ET AL: "Good or evil: CD26 and HIV infection" JOURNAL OF DERMATOLOGICAL SCIENCE, vol. 22, no. 3, April 2000 (2000-04), pages 152-160, XP002302250 ISSN: 0923-1811 the whole document</p>	1-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/050753

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9745117	A	04-12-1997	AU 3224997 A CA 2256669 A1 CZ 9803893 A3 EP 0914123 A1 WO 9745117 A1	05-01-1998 04-12-1997 14-07-1999 12-05-1999 04-12-1997
WO 0071571	A	30-11-2000	AU 6559100 A BG 106096 A BR 0010564 A CA 2369933 A1 CN 1374969 T CZ 20014097 A3 EE 200100600 A WO 0071571 A2 EP 1180116 A2 HU 0202367 A2 JP 2003500417 T NO 20015536 A NZ 516075 A PL 351680 A1 SK 16452001 A3 US 6613879 B1 ZA 200109151 A	12-12-2000 31-05-2002 19-02-2002 30-11-2000 16-10-2002 13-02-2002 17-02-2003 30-11-2000 20-02-2002 28-11-2002 07-01-2003 10-01-2002 26-03-2004 02-06-2003 05-03-2002 02-09-2003 17-12-2002
US 5962216	A	05-10-1999	BE 1008580 A3 BE 1008581 A3 AU 694546 B2 AU 3248695 A WO 9605863 A1 CA 2203622 A1 EP 0769967 A1 JP 10508291 T NO 970748 A NZ 291368 A US 2002160943 A1 US 6342480 B1	04-06-1996 04-06-1996 23-07-1998 14-03-1996 29-02-1996 29-02-1996 02-05-1997 18-08-1998 10-04-1997 29-04-1999 31-10-2002 29-01-2002
WO 9967278	A	29-12-1999	DE 19828113 A1 AU 766726 B2 AU 4900799 A BR 9911468 A CA 2335992 A1 CN 1306540 T WO 9967278 A1 EP 1087991 A1 HU 0102281 A2 JP 2003524591 T NO 20006484 A NZ 508722 A PL 345151 A1 RU 2226533 C2 US 2004171555 A1 US 2002049164 A1	05-01-2000 23-10-2003 10-01-2000 20-03-2001 29-12-1999 01-08-2001 29-12-1999 04-04-2001 28-11-2001 19-08-2003 19-12-2000 25-07-2003 03-12-2001 10-04-2004 02-09-2004 25-04-2002
WO 03048190	A	12-06-2003	WO 03048190 A2	12-06-2003
WO 9967254	A	29-12-1999	AU 771780 B2 AU 4828099 A	01-04-2004 10-01-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/050753

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9967254	A	AU 4828199 A	10-01-2000
		CA 2336160 A1	29-12-1999
		EP 1088098 A2	04-04-2001
		JP 2002518063 T	25-06-2002
		WO 9967417 A2	29-12-1999
		WO 9967254 A2	29-12-1999